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Electroencephalography of Premature Infants

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Declaration

The thesis submitted is the candidate's own work and has not been submitted for another degree, either at University College Cork or elsewhere.

______________________ 04/09/2020

Rhodri Lloyd ______________________

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Thesis Abstract

Background and Objectives

The early prediction of neurodevelopmental outcome in very preterm infants remains challenging. An objective tool with the potential to provide useful information about preterm brain health is the electroencephalogram (EEG) but current knowledge remains incomplete in infants of this age group. The ability to record continuous conventional EEG is usually overlooked due to the ease of application and maintenance of the amplitude-integrated EEG (aEEG). The aEEG is routinely used to identify seizures, assess background EEG and predict outcome, despite the fact that it has considerable limitations for preterm infants in particular. Research using conventional multichannel EEG in preterm infants is ongoing but studies tend to be of short duration and at varying periods post-birth. To progress and achieve future information about the predictive ability of EEG for neurodevelopmental outcome in preterm infants, more in-depth analysis is required.

In this thesis, I aim to progress current knowledge in very preterm infants <32 weeks gestational age (GA) by investigating the ability of EEG to assess neurological wellbeing and to predict neurodevelopmental outcome at 2 years. Furthermore, I aim to investigate and described the frequency and characteristics of electrographic seizures during the early postnatal period in very preterm infants, and compare this to the existing literature. In addition, I aim to develop a standardised scheme for assessing both the normal and abnormal EEG features of preterm infants according to post-menstrual age. Finally, I aim to investigate the EEG of preterm twins and assess EEG concordance between monochorionic-diamniotic (MCDA) and dichorionic-diamniotic (DCDA) twins.

Methods

Two cohorts of preterm infants <32weeks GA were recruited from 2009-2014 (cohort 1; 2009 – 2011 and cohort 2; 2013 – 2014). All infants had continuous conventional video-EEG monitoring. The EEGs from cohort 1 were recorded as soon as possible after birth, while the EEGs from cohort 2 were recorded within the first 12 hours of age, continued for approximately 72 hours with further short follow-up recordings at 32 weeks corrected

gestational age and at pre discharge. EEG was graded as normal (normal or mildly abnormal) and abnormal (moderately abnormal or severely abnormal). Clinical demographics, clinical risk scores and details of the clinical course in the Neonatal Intensive Care Unit (NICU) were also collected. Neurodevelopmental outcome was assessed at 2 years of age via the Bayley Scales of Infant Development-III (Bayley-III).

We used cohort 1 to develop a multimodal model for the prediction of neurodevelopmental outcome. This model incorporated simultaneous multi-channel electroencephalography (EEG), peripheral oxygen saturation (SpO_2), and heart rate (HR) recordings. One-hour epochs of EEG, HR and SpO_2 were then extracted at 12 and 24 hours of age from each recording. EEG grades were combined with GA and quantitative features of HR and SpO_2 in a logistic regression model to predict outcome. Clinical status was also incorporated into the model to predict neurodevelopmental outcome.

EEGs from both cohorts were used to examine seizures in very preterm infants. The entire video-EEG recording for each infant was reviewed and all electrographic seizures were visually identified, annotated, and analysed. Quantitative descriptors of the temporal evolution of seizures were calculated including total seizure burden, mean seizure duration, and maximum seizure burden. For each seizure, the onset location, morphology and evolution were described.

We used cohort 1 to develop a standardised EEG assessment scheme for preterm infants. Initially a comprehensive literature review was performed by two electroencephalographers (EP & RL[1]) to identify existing descriptions and definitions of both normal and abnormal EEG features of preterm infants. This was followed by development and testing phases of a new standardised EEG assessment scheme. Two neonatal EEG experts, not involved in the development phase of the study then evaluated the scheme using random 2-hour EEG epochs from 24 infants <32 weeks GA. Where disagreements were found between both experts, the features where further checked and modified. Finally, both experts used the scheme to

[1] Elena Pavlidis and Rhodri Lloyd

independently evaluate 2-hour EEG epochs from 12 additional infants <37 weeks GA. Percentage of agreement between observers were calculated.

In the penultimate study, using infants from cohort 2, the concordance between continuous video-EEG recordings in preterm twin pairs was examined. EEGs commenced almost synchronously in twin pairs and continued until the infants were approximately 72 hours of age, while additional, shorter recordings at 32 weeks and post discharge were also recorded in unison. Visual EEG interpretation was assessed using standardised criteria from the previous chapter. Correlations were estimated within twin pairs and compared to age-matched singletons. Additionally, quantitative, mathematical EEG features were extracted and generated to represent EEG power, discontinuity, and symmetry. While controlling for GA, intra-class correlations (ICC) estimated similarities within twins.

For the final study, EEGs from cohort 2 at 3 time-points over the neonatal course was used. EEGs were reviewed and scored by two electroencephalographers (EP & RL) based on the newly developed standardised EEG assessment scheme, which considered normal and abnormal activity. Bayley-III assessed neurodevelopmental outcome at 2 years corrected age.

Results

Data from forty-three infants, from cohort 1, were used to develop a multimodal model for the prediction of 2-year neurodevelopmental outcome. Twenty-seven infants had good outcomes and 16 had poor outcomes or died. While performance of the model was similar to a clinical course score graded at discharge, with an area under the receiver operator characteristic (AUC) of 0.83 (95% confidence interval, CI: 0.69 – 0.95) for the physiological model vs 0.79 (0.66 – 0.90) (p=0.633) for the clinical course score, the model was able to predict 2-year outcome days after birth. Although the differences failed to reach statistical significance, the model did have a larger AUC compared to the individual physiological features, highlighting the potential value of multimodal monitoring during the transitional period.

After visually analysing 6,932 hours of EEGs from 120 preterm infants from both cohorts, we identified that 6 infants (5%, 95% CI: 1.9% to 10.6%) had electrographic seizures in the first 3 days. Median (interquartile range, IQR) total seizure burden, mean seizure duration, and maximum seizure burden were 40.3 (5.0, 117.5) minutes, 49.6 (43.4, 76.6) seconds and 10.8 (1.6, 20.2) minutes/hour respectively. Seizure burden was highest in two infants with significant abnormalities on neuroimaging.

In the final analysis of the EEG assessment scheme, good percentage agreements were obtained from all patients and EEG feature categories. Median agreements of between 80% and 100% were identified from the 4 categories. No difference was found in agreement rates between the normal and abnormal features ($p = 0.959$), neither between the younger preterm groups (<30 weeks GA) and the older preterm group (>30 weeks GA), ($p = 0.249$).

The twins study saw the recruitment of 10 twin pairs, four monochorionic diamniotic (MCDA) and six dichorionic diamniotic (DCDA) pairs, and 10 age-matched singleton pairs. For the MCDA twins, 17/22 mathematical EEG features had significant (>0.6; $p<0.05$) ICCs at one or more time-points, compared to 2/22 features for DCDA twins and 0/22 features for singleton pairs. For the MCDA twins, all 10 features of discontinuity and all four features of symmetry were significant at one or more time point. Three features of the MCDA twins (spectral power at 3 – 8 Hz, skewness at 3 – 15 Hz, and kurtosis at 3 – 15 Hz) had significant ICCs over the course of all three time-points. No features for the DCDA group or control singleton pairs had significant ICCs over all three time-points.

For the final study, 57 infants were included to establish whether serial multichannel video-EEG has a role in predicting 2-year outcome. From the 57 infants included, 40 had good outcome and 16 had poor outcome or died. All three serial EEGs were individually predictive of abnormal outcome, with AUCs of 0.68 (95% CI: 0.55 – 0.80); 0.84 (0.73 – 0.94); and 0.91 (0.83 – 1), ($p<0.001$). Comparatively, the predictive value (AUC) for a poor clinical course was 0.68 (0.54 – 0.80), while the presence of Intraventricular Haemorrhage (IVH) grade III/IV or cystic Periventricular Leucomalacia (cPVL) was 0.58 (0.41 – 0.75), ($p=0.342$).

Conclusions

This research has utilised continuous conventional video-EEG of very preterm infants during the early postnatal period to improve understanding of early brain function and its relationship with future neurodevelopmental outcome. I have shown that quantitative analysis of multimodel preterm physiological signals, has the potential to predict mortality or delayed neurodevelopment at 2 years of age. Further studies with increased numbers are required to confirm the observed results.

I identified that electrographic seizures are infrequent within the first few days of birth in very preterm infants and that we report a smaller seizure frequency than previous studies in similar cohorts. Seizures in this population are difficult to detect accurately without continuous multichannel EEG monitoring. This is the first study to use continuous, long duration, video-EEG monitoring to qualitatively and quantitatively describe electrographic seizures in preterm infants <32 weeks during the early postnatal period.

In addition, for the first time, I have developed and described a standard EEG assessment scheme specifically for very preterm infants. When implemented, this showed good interobserver agreement. This can provide important information to NICU staff about normal or abnormal brain activity, maturation and neuromonitoring during critical care.

I report the first study to investigate the EEG of very preterm twins during the early postnatal period. Preterm twin EEG similarities are subtle and difficult to identify visually, however this is clearly evident through quantitative analysis. MCDA twins showed stronger EEG concordance across all time-points, thus confirming a strong genetic influence on preterm EEG activity at this early stage of development.

Finally, in a prospective study investigating EEG for the prediction of neurodevelopmental outcome, I have shown using serial multichannel EEG recordings that the pre-discharge EEG was the best predictor of 2-year outcome.

This thesis has progressed the state of the art in preterm EEG, paving the way for further conventional EEG studies that use a standardised EEG assessment scheme. This study has also shown that seizure in preterm infants are infrequent in the early postnatal period and that there is high EEG concordance between some twin pairs. An EEG pre-discharge may be the best predictor of 2-year neurodevelopmental outcome. The assessment scheme was developed and its ability to predict 2-year outcome should now be validated in a large scale multicentre study.

Abbreviations

AS	Active Sleep
AED	Anti -Epileptic Drug
aEEG	Amplitude-integrated Electroencephalography
ASA	Acute Stage Abnormalities
AUC	Area Under the received operator Curve
Bayley-III	Bayley Scales of Infant Development-III
BPD	Bronchopulmonary Dysplasia
BW	Birth Weight
CBF	Cerebral Blood Flow
CI	Confidence interval
cGA	corrected Gestational Age
Cl^-	Chloride ion
CLD	Chronic Lung Disease
CO2	Carbon Dioxide
CONS	Coagulase-Negative Staphylococci
CP	Cerebral Palsy
CPAP	Continuous Positive Airways Pressure
cPVL	Cystic Periventricular Leucomalacia
CRIB	Clinical Risk Index for Babies
CSA	Chronic Stage Abnormalities
CTG	Cardiotocography
CRUS	Cranial Ultrasound
DC	Direct Current
DCDA	Dichorionic-Diamniotic
Dz	Dizygotic
E.coli	Escherichia coli
EEG	Electroencephalogram
ELBW	Extremely Low Birth Weight
EOS	Early Onset Sepsis
EPSPs	Excitatory Postsynaptic Potentials
GA	Gestational Age
GABA	Gamma-aminobutyric Acid
HIE	Hypoxic Ischaemic Encephalopathy
HR	Heart Rate
IBI	Interburst Interval
ICC	Intra-class Ccorrelations
IPSPs	Inhibitory Postsynaptic Potentials
IPH	Intraparenchymal Haemorrhage
IQR	Interquartile Range
IUGR	Intrauterine Growth Restriction
IVH	Intraventricular Haemorrhage
LOS	Late Onset Sepsis
MCDA	Monochorionic-Diamniotic

MRI	Magnetic Resonance Imaging
Mz	Monozygotic
Na+	Sodium ion
NEC	Necrotising Enterocolitis
NICE	National Institute for Health and Care Excellence
NICU	Neonatal Intensive Care Unit
NIRS	Near-infrared Spectroscopy
NPV	Negative Predictive Value
OR	Odds Ratio
PLEDS	Periodic Lateralized Epileptiform Discharges
PMA	Post-menstrual Age
PPV	Positive Predictive Value
PROM	Preterm Rupture of Membranes
PPROM	Prolonged Premature Rupture of Membranes
PRS	Positive Rolandic Sharps
PTS	Positive Temporal Sharps
PTT	Premature Temporal Theta
QS	Quiet Sleep
r	Pearson's correlation coefficient
RDS	Respiratory Distress Syndrome
ROP	Retinopathy of Prematurity
RR	Respiratory Rate
SAD	Slow Anterior Dysrhythmia
SAT	Spontaneous Activity Transient
SCORE	Standardized computer-based organised reporting of EEG
SpO2	Peripheral Oxygen Saturation
SROM	Spontaneous Rupture of Membranes
STOPS	Sharp Theta on the Occipitals of Prematures
tABP	Total Absolute Band Power
VLBW	Very Low Birth Weight
WMI	White Matter Injury

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Presentations

- Congress of the European Academy of Paediatric Societies, EAPS 2016 Geneva, Switzerland – E-poster presentation: Lloyd RO, O'Toole JM, Livingstone V, et al. Correlation of EEG within preterm twin infants: visual and quantitative analysis.
- Brain Monitoring and Neuroprotection International Conference 2015 – 2nd prize Oral: Lloyd RO, O'Toole JM, et al. Electrographic seizures during the early postnatal period in preterm infants less than 32 weeks.
- INFANT & College of Medicine & Health (COMH) Research Day2015– Poster Presentation: Lloyd RO, O'Toole JM, et al. Electrographic seizures during the early postnatal period in preterm infants less than 32 weeks.
- Brain Monitoring and Neuroprotection International Conference 2014, Florida, USA – Poster Presentation: Lloyd R, Goulding R, Filan P, Boylan G. Overcoming the practical challenges of electroencephalography for very preterm infants in the neonatal intensive care unit. 8th International Conference on Brain Monitoring and Neuroprotection in the Newborn.
- Irish Society of Clinical Neurophysiology Scientific Meeting, Dublin, Ireland 2014– Oral presentation - Presentation: Overcoming the practical challenges of electroencephalography for very preterm infants in the neonatal intensive care unit.
- INFANT & College of Medicine & Health (COMH) Research Day2016– Poster Presentation: Lloyd RO, O'Toole JM, Livingstone V, et al. Correlation of EEG within preterm twin infants: visual and quantitative analysis.

Thesis Structure

This thesis begins with an introduction to prematurity, the normal and abnormal EEG features of preterm infants, what is known about seizures in preterm infants and the prognostic value of aEEG/EEG in this group. The second chapter presents the methodology implemented for the studies undertaken as part of this thesis, followed by five chapters of research studies based on the premature EEG and the final conclusion chapter.

Chapter 1 introduces the thesis by describing prematurity and how early birth influences brain development and how it is associated with early neonatal death, neonatal morbidity and adverse developmental outcomes. The normal progression of fetal brain development can be disrupted by premature birth. This chapter delves into the pathophysiology of the fetal brain at different developmental stages. Premature birth is discussed, including delivery, resuscitation, multiple pregnancies, initial assessments, neonatal complications, current imaging and monitoring techniques and also treatment. Normal and abnormal aEEG/EEG features are described in detail: what is expected at different GA, how these features differ at different GA, and what influences the preterm EEG. In addition, to describing abnormal aEEG/EEG and how it relates to short term outcome, the frequency of seizures and the prognostic value of aEEG/EEG in preterms is described. Finally, I described the neurodevelopmental assessments used during childhood, with the emphasis on the Bayley Scales of Infant and Toddler Development–III assessment which was utilised implemented in this thesis.

Chapter 2 details the general methodology used for all the studies within the thesis. In this chapter, the retrospective and prospective cohorts are described, in addition to the processes used for ethical approval, study protocol and parental consent. The chapter describes in detail the neonatal EEG electrode application methodology used in this cohort in the NICU environment. Finally, methods used for data collection, visual analysis, artefact identification and assessment of developmental outcome is described.

Chapter 3 evaluates the prognostic performance of a multimodal model incorporating physiological signals and clinical information in preterm infants during the first 24 hours of life, to assess 2-year outcome.

Chapter 4 identifies the frequency of seizures in preterm infants during the first 3 days of life, while also reporting quantitative descriptors of the temporal evolution of seizures, such as total seizure burden.

Chapter 5 describes the development of a new standardised assessment scheme for evaluating both normal and abnormal EEG features in preterm infants.

Chapter 6 describes concordance within the EEGs of preterm twins, while using visual and mathematical analysis.

Chapter 7 evaluates the prognostic performance of the new assessment scheme in preterm infants with serial EEGs, to predict neurodevelopmental outcome at 2 years of age.

Chapter 8 describes the significance and implications of the main findings emerging from the thesis. Limitations of the research and suggestions for future work are reported to provide a platform for future research.

Chapter 1. Introduction

1.1. Prematurity

Preterm birth occurs when infants are born before 37 weeks gestation (1). Approximately 50% of premature births occur between 35 and 36 weeks (2). However, births can occur at a moderately preterm stage (32 – 34 weeks), very preterm stage (28 – 32 weeks), or at an extremely preterm stage (earlier than 28 weeks). 7% of all live births in the UK are born before 37 weeks (3) and the very and extremely preterm birth rate in the UK and Ireland, has recently been reported as approximately 1% of all total births (4). In America, the rate of extremely preterm birth is less than 1% of all births and 6% of all preterm birth (5). The earlier the birth, the more susceptible the infant will be to health complications (1).

In 2010, 14.9 million (11%) of all births worldwide were premature, and 28% of all early neonatal deaths were directly related to prematurity (6, 7). In several European countries, the estimate was closer to 5%, while certain African countries was as high as 18% (7). As well as neonatal mortality, high rates of neonatal morbidity and adverse development, such as cerebral palsy (CP) and learning difficulties, are associated with premature birth (1, 6). Specific complications such as intraventricular haemorrhage (IVH), necrotising enterocolitis (NEC), retinopathy of prematurity (ROP), neonatal infections, and chronic lung disease (CLD) are serious complications of extreme prematurity in the developed world (8). Although the cause of preterm birth is still unclear, several factors show an association: genetic disorders, environmental exposure, infertility treatments, preeclampsia, infection, chorioamnionitis IUGR, and other medical conditions of the fetus or mother (1, 9, 10). Furthermore, smoking and the use of recreational and illicit drugs during pregnancy increases the probability of preterm birth (11). Reports suggest that 30% of prematurity is associated with preterm rupture of membranes (PROM) (1, 12), 15 – 20% is due to detected medical issues, while 50% is spontaneous and of unknown nature, referred to as an idiopathic preterm birth (1, 13). It is suggested that maternal stress could be a factor impacting spontaneous birth (12).

1.2. Fetal Brain Development

1.2.1. Gastrulation & Neuroectodermal Progenitor Cells

Post-conception, the initial development of the embryo takes place with the formation of the neural plate. By the second week post conception the embryo is a simple two layered oval structure. Over the next week the embryo is transformed during gastrulation into 3 layers. From the ectodermal layer emerge the neuroectodermal progenitor cells which will give rise to the brain and spinal cord. These progenitor cells line up along the rostro-caudal axis to form the neural plate (14). Figure 1-1 illustrates the developmental timeline of the neonatal brain.

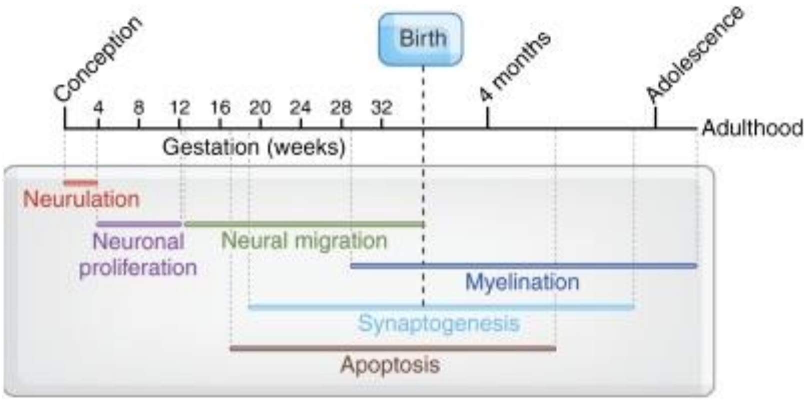


Figure 1-1 Normal brain development timeline of brain circuits ; reprinted from Tau GZ and Peterson BS (15).

1.2.2. Neurulation

Neurulation proceeds from 3-4 weeks as the neural plate gradually grows in length and begins to fold onto itself, along the rostrocaudal axis. At 6 weeks the folding of the neural plate closes in on itself and fuses to form the neural tube, which in turn gives rise to the central nervous system (16). Following this the neural crest cells are formed, and these will eventually develop into the dorsal root ganglia, sensory ganglia, autonomic ganglia and schwann cells (14). The neural tube then starts to bulge and form three main parts: the forebrain, midbrain and hindbrain. The forebrain forms the limbic system, the thalamus, the hypothalamus, the basal ganglia, and the cerebral cortex. The hindbrain eventually forms the medulla, cerebellum and pons, with a rear part of the hindbrain eventually forming the spinal cord (17). All of this regional patterning is genetically driven and dependent on a complex interplay of signalling molecules and their concentration gradients (18). During this period, the progenitor cells lie next to an area that eventually becomes the ventricles, therefore this area is known as the ventricular zone.

At approximately 8 weeks, three vesicles expand from the anterior end of the neural tube, namely prosencephalon, mesencephalon and rhobencephalon. Furthermore, prosencephalon and rhobencephalon subdivisions occur which leads to the development of 5 vesicles. The prosencephalon subdivides into the telencephalon and the diencephalon, while the rhombencephalon subdivides into the metencephalon and the myelencephalon. The telencephalon will become the cerebrum, the diencephalon will become the thalamus and the hypothalamus, while the midbrain remains an established brain region from the primary vesicle development stage (19). The development of the embryonic brain regions to brain structures is evident in figure 1-2.

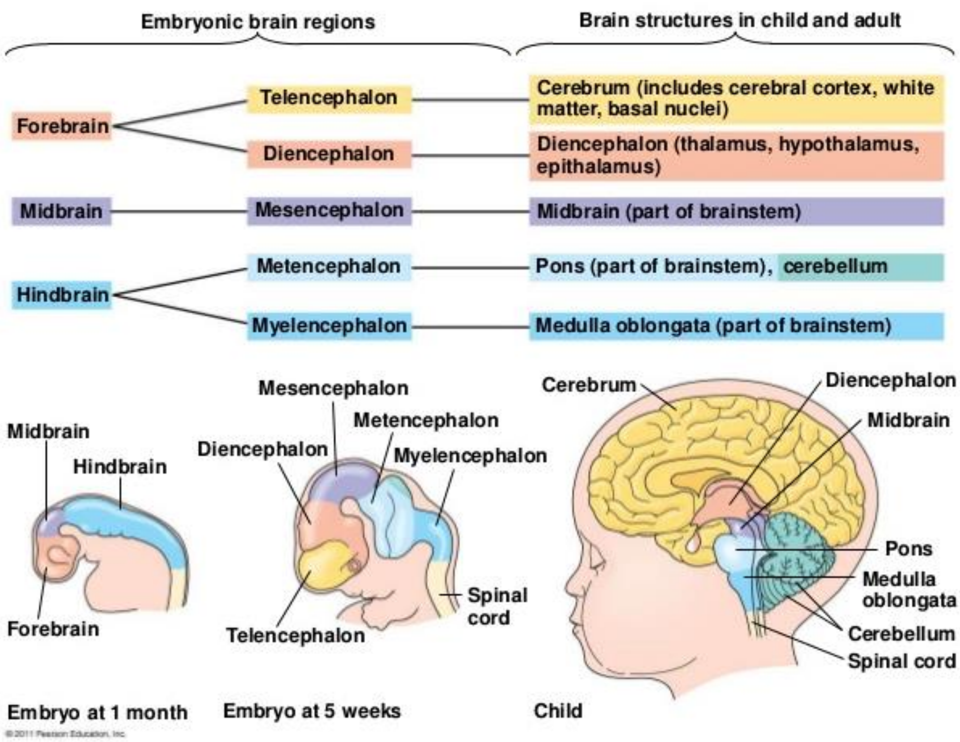


Figure 1-2 Brain development from embryo to child (http://www.slideshare.net/qinlee/48-49-22255847)

1.2.3. Neuronal Proliferation

The brain and nervous system begin to form at an early stage, with neural cells migrating to create millions of neurons. This is known as neuronal proliferation, a rapid development that continues between the 4^{th} and 12^{th} week of gestation (15). The progenitor cells at the ventricular zone produce billions of neurons, which migrate and terminate in the neocortex.

1.2.4. Neuronal Migration

At approximately 12 weeks of gestation, glial cells begin to develop and neuronal migration also occurs. This is a time when neurons migrate from the ventricular zone to specific regions of the brain, eventually forming synapses and ensuing networks of neural connectivity (16). There are two types of neuronal migration, namely radial and tangential migration. In the cerebrum, radial migration of neurons originate from the ventricular and subventricular zones and give rise to the projection of the cerebral cortex and deep nuclear structures. Tangential migration differs, by giving rise to the interneurons of the cortex. Alternatively in the cerebellum, radial migration forms the purkinje cells and dentate nucleus, while the tangential migration forms the external granular layer, before migrating radially to form the internal granule cells of the cerebellar cortex (14). Early migrating neurons from the ventricular zone form the preplate. These divide to form the marginal zone and the subplate. Neurones that migrate later use the chemical Reelin from the marginal zone to form the characteristic 6 layers of the neocortex and stop migrating further. Neurones terminate and differentiate into multiple types of neurones in the cortex (Figure 1-3).

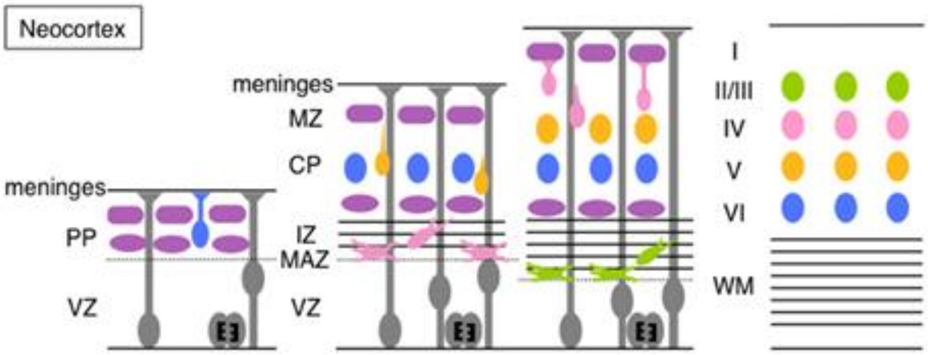


Figure 1-3 Neuronal Migration. Neurones migrate from the ventricular zone (VZ) to form the preplate (PP). The PP differentiates to form different layers; including cortical plate (CP) and marginal zone (MZ), before regressing and forming a mature cortex of six layers. Reprinted from Hayashi et al. (20)

1.2.5. Neuronal Organization

At approximately 20 weeks of gestation, numerous important organizational events begin. The subplate zone thickens and functions as an area where afferent fibres wait before their ingrowth into the cortical plate and corticothalamic processes also grow down into the subplate from layer 5/6 of the cortex. Accumulation of thalamocortical afferents in the subplate zone enables synaptic interaction. This process is called synaptogenesis, which refers to formation of synapses throughout the brain and establishing its processes. Synapses consist of the axonal presynaptic membrane and dendritic post synaptic membrane, which allows nerve impulses to transmit from one neuron to another, relaying information via electrical and chemical processes. The action potential initially arrives at the axon terminal and causes a release of neurotransmitters into the synaptic cleft, which then binds to a post-synaptic receptor and opens specific ion channels, allowing the action potential to transmit down the postsynaptic neuron. The first thalamocortical synapses develop in the cortical plate and continues to develop, which in turn creates the synapse-rich subplate zone (21). Synaptogenesis continues until adolescence, but peaks at 34 weeks GA, where approximately 40,000 synapses are created per second (15). Apoptosis is another important event, also known as programmed cell death, which is the deliberate death of unwanted cells, mediated by an intracellular program. This is an important process that creates functional circuits and pairs presynaptic and post-synaptic target neurons while also ensuring that the population of neuronal and glial cells is balanced, with the right amount of cells available (22). This numerical balance of neurons consequently leads to apoptosis of neurons that failed to connect. Approximately 40 – 75% of all neurons created do not survive, while the survivors maintain neurotophic factors and manage to synapse. Failure of the apoptotic process would lead to the persistence of mutated cells, causing major brain malformations, autoimmune disease, and cancer (14). By the 34th week, the thickness of the subplate zone is decreased, while ingrowth of the callosal and long cortico-cortical pathways into the cortex occurs.

1.2.6. Myelination

The next maturational process is myelination, where the myelin sheath grows around a nerve to increase the propagation speed of the action potential down the axon (Figure 1-4). The myelin sheath acts as an insulator that increases the velocity of the impulse ensuring rapid electrical transmission (23). Myelination initially begins in the peripheral nervous system, at the motor root of the neurone, then shortly begins in the sensory roots. Thereafter, components of the central nervous system undergo myelination (14). Cortical myelination begins in the central cortical areas around the central sulcus, followed by myelination of the occipital poles, before myelination of the frontal poles (14).

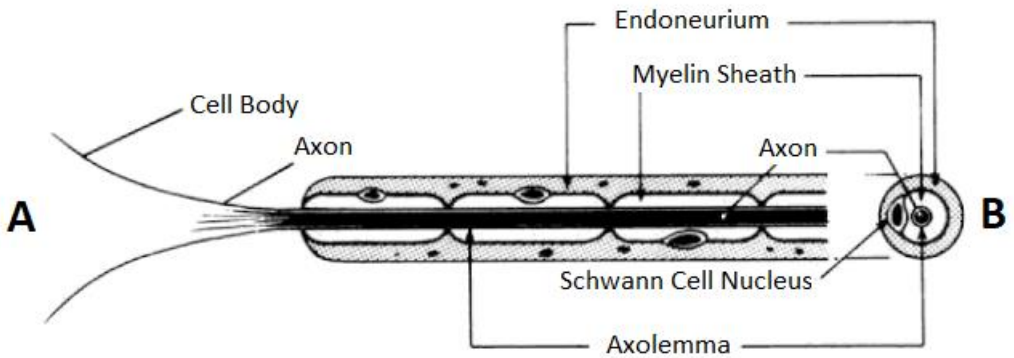


Figure 1-4 A longitudinal and transverse section of a nerve fibre ; longitudinal (A) and transverse (B) (24).

This myelination process begins approximately during 26 – 28 weeks and continues into adult life, but peaks during the first 2 years of life.

1.3. Preterm Labour and Birth

Preterm birth occurs when fetal brain development is at an early stage. As a result, preterm infants are at greater risk of neurodevelopmental complications, such as motor or cognitive disabilities. The risk or incidence of morbidity and mortality is higher in the more premature infants (1).

Preterm labour can occur spontaneously or be induced for medical reasons. The occurrence of spontaneous labour can derive from prolonged preterm rupture of membranes (PPROM) or cervical incompetence (where the cervix begins to dilate before pregnancy reaches term) (25). PPROM occurs when the amniotic membranes of the amniotic sac rupture prior to 37 weeks of gestation, releasing the amniotic fluid and leading to the onset of labour (26). Occasionally, these events cannot be explained, although certain factors such as placental abruption, antepartum haemorrhage, cervical incompetence, stress or infection can be factors (25). Various antenatal chronic uteroplacental insufficiencies, including underlying IUGR, pre-eclampsia, abnormal fetal cardiotocography (CTG), or multiple pregnancies, can lead to an induced labour (27). Multiple pregnancy, (two or more foetuses) is also associated with preterm birth. Approximately 15 – 20% of preterm infants are from multiple pregnancies, while nearly 60% of twins are born prematurely (28).

1.3.1. Multiple Pregnancies

Twins can be categorised as either monozygotic or dizygotic. Dizygotic twins are created when two different eggs are fertilised by two different sperm. Each fetus has a separate amniotic sac and placenta within the uterus at the same time. They are classified as dichorionic-diamniotic, and are referred to as non-identical.

Monozygotic twins are identical twins, which develop from a single ovum following fertilization by one sperm. This shared zygote then splits within days resulting in two embryos. If the split occurs quickly, they can also become dichorionic-diamniotic (2 placentae and 2 sacs), which occurs at a rate of 25% in monozygotic twins. The other 75% are monochorionic (1 placenta), where the zygote splits gradually (29). There are two types of monochorionic twins. Monochorionic-diamniotic twins occur when the zygote splits at days 4 – 8, resulting in an amniotic sac for each twin. Monochorionic-monoamniotic twins occur when the zygote splits later, at days 8 - 13, resulting in a shared amniotic sac. Generally, chorionicity is regarded as being of more interest than zygosity, as risk of early pregnancy loss increases with monochorionic placentation (30).

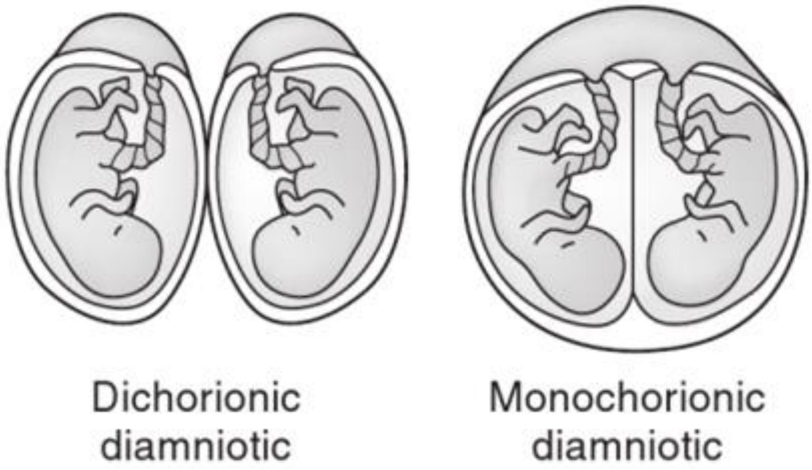


Figure 1-5 Dichorionic diamniotic and Monochorionic diamniotic twins in the placenta; reprinted from Fox, H Pathology of the placenta (31)

1.3.2. Delivery

The mode of delivery depends on the condition of the fetus, the mother and also the GA of the fetus. Signs of fetal distress, such as severely abnormal CTG or Doppler readings, may lead to an urgent caesarean section (c-section) (32). C-section is a surgical incision of the maternal abdomen and uterus to create space for the baby to be delivered through the abdomen. Even though it is now a common surgery that is relatively safe and used frequently, it still includes risks. In addition to the abnormal CTG, placental abruption, uterine rupture or prolapse cord may also lead to an emergency c-section (33). In certain situations, C-sections can also be planned in advance e.g. when a baby is in the breech (bottom down) or transverse (sideways) positions (34). Additionally, severe pre-eclampsia, previous caesarean section, and complicated multiple pregnancies (e.g presentation positions) can also lead to elective sections (34).

1.3.3. Resuscitation

Following birth, the infant is wrapped and kept warm and then be transferred to a trolley for resuscitation, if necessary. The aim of resuscitation is to support breathing, circulation, temperature control and to ensure airway patency (35). As preterm infants may have immature lungs and are susceptible to hyaline membrane disease or respiratory distress syndrome (RDS), establishing regular breathing may be difficult, therefore respiratory support is usually provided. Possible options include continuous positive airways pressure (CPAP), intubation or by providing surfactant (36). CPAP supplies constant air pressure, via the nostrils, to help keep the airways alveoli open (36). Intubation is a procedure that requires the placement of a tube in the airway to allow an open airway and ensure that both the trachea and lungs are protected from the aspiration of stomach contents (27). Finally, surfactant is given within the first 2 hours after birth, due to the lack of naturally produced surfactant in the immature lungs, stabilising the small alveoli air sacs in the lungs and reducing the surface tension of fluid in the lungs (36, 37).

1.3.4. Assessment at birth – Apgar Score

In 1952, Dr Virginia Apgar designed a scoring method to evaluate the general clinical condition of infants at birth. The 'Apgar Score' is now universally recognised as a method which can provide a quick, simple indication of the infant's condition. All infants are immediately assessed at birth, with specific clinical features considered in a structured way. Five measurable features are graded to generate an Apgar score: skin colour, heart rate, reflex irritability, muscular tone, and respiration (38). Each feature scores from 0 to 2 (Table 1-1). A total score of 10 can therefore be acquired. The scores are generally performed twice at 1 and 5 minutes, but may be recorded up to 20 minutes post-birth, in situations such as prolonged resuscitation.

CRITERIA		SCORE		
		0	1	2
Skin Colour	***A**ppearance*	Pale or Blue all over	Blue hands and feet	Completely pink
Heart Rate	***P**ulse*	Absent	< 100 beats per minutes	> 100 beats per minutes
Reflex irritability (response to stimulation)	***G**rimace*	Absent	Only facial movements	Cough, sneeze, cry, or pulls away
Muscular tone	***A**ctivity*	Absent (Floppy)	Flexion of arms & legs	Active and spontaneous
Respiration	***R**espiration*	Absent	Slow or irregular with weak cry	Good breathing rate & crying

TABLE 1-1 APGAR SCORING SYSTEM.

Although developed in the 1950s, today the Apgar scoring system is still used in general neonatal practice (39). Some limitations have however become apparent over the years. Subjectivity is regarded as the most problematic limitation of the Apgar test, where large inter-observer variability can occur. In addition, several other factors can influence the score, such as GA, congenital malformations, maternal sedation and trauma (40). Its role in preterm infants is questionable, as their low scores might be based on their immaturity, even if relatively healthy for their gestation (41).

1.4. Preterm Infants in the NICU

Once the preterm infant less than 32 weeks is stable and receives effective respiratory support, they are transferred from the delivery suite to the neonatal intensive care unit (NICU), for ongoing intensive care. Respiratory support persists, while heart rate, blood pressure and temperature are constantly monitored. In most situations, preterm infants less than 32 weeks will be put in an incubator which stabilises temperature and decreases infection risk. Early clinical assessments are undertaken to assess the condition of the infant.

1.4.1. Assessment at birth – CRIB II Score

Clinical Risk Index for Babies II (CRIB II) is a clinical risk assessment tool used to assess the baby's medical condition in the first hours of birth (42). This is calculated based on five items: gender, GA, birth weight (BW), admission temperature, and the base deficit in the first blood sample taken. This is a scoring system that was adjusted from a previous version (CRIB) score in 1993. CRIB II scores can range from 0 to 27, with lower scores indicating a better prognosis. It is a useful tool for predicting mortality in low BW infants, with a score of ≥11 predictive of mortality and morbidity (sensitivity: 83 - 95% and specificity: 82 - 84%) (43). The first CRIB version included BW, GA, maximum and minimum fraction of inspired oxygen and maximum base excess during the first 12 hours, and congenital malformations (44). However, the applicability of the CRIB score in modern neonatal intensive care warranted a revision of this scoring system. The primary concern was that the 12 hour window was too long, allowing time for early treatment to affect the infants state (42). There are still some limitations to CRIB II, namely the fact that it is an epidemiologic tool, while perinatal conditions with adverse consequences are not considered (45), however it does consider the medical condition at an early stage allowing for a quick and simple overall assessment of the infants condition.

Unfortunately, critical complications can occur in preterm infants during their stay in the NICU, which can often last weeks or months. These complications include intraventricular haemorrhage (IVH), periventricular leucomalacia (PVL), infection, necrotizing enterocolitis (NEC), chronic lung disease (CLD) and retinopathy of prematurity (ROP). These conditions can have an adverse effect on the neurodevelopment of preterm infants and increases the risk of adverse long-term outcome (46).

1.4.2. Complications of prematurity

1.4.2.1. Intraventricular Haemorrhage (IVH)

IVH is when bleeding occurs inside the lateral ventricles of the brain. The incidence is dependent on GA, with a higher risk of IVH in the most premature infants (14, 47). IVH most commonly occurs during the first three days of postnatal life (48). Although neonatal care has improved, incidence of any grade IVH still ranges between 13 – 20% (49, 50), and appears more prominent in extremely preterm infants with an incidence of closer to 45% (50-53).

IVH originates in the germinal matrix. This is a vascularised area in the floor of the lateral ventricle and a source of glial precursor cells and capillaries. The immature capillaries are fragile, due to the lack of muscular and collagen support (51). The immature brain does not have mature autoregulation of cerebral circulation to control blood pressure changes (54), therefore increased and decreased alteration of cerebral blood flow pressure can cause the ependymal layer to rupture and bleed into the ventricle (51).

The classification scheme used to grade the degree of an IVH is the Papile classification (Table 1-2). This system is composed of four grades and has been modified by Volpe (14, 55).

Papile Grading	Volpe Grading	
Grade I	Germinal Matrix Haemorrhage/ Subependymal Haemorrhage	Isolated haemorrhage in the germinal matrix
Grade II	Grade II	Haemorrhage inside the ventricle (10-50% of ventricular area), however doesn't have enough blood to dilate the ventricle.
Grade III	Grade III	Haemorrhage inside the ventricle (>50% of ventricular area) with enough blood to distend the ventricle.
Grade IV	Severe periventricular haemorrhagic infarction / Intra-parenchymal haemorrhage	Dilation of germinal matrix can impair venous drainage from the terminal vein and result in venous infarction.

TABLE 1-2 COMBINED PAPILE AND VOLPE GRADING CLASSIFICATIONS.

Cranial ultrasound (CRUS) is a tool used to detect IVH. It is recommended that infants <32 weeks GA or of <1500g birthweight, should have serial CRUS during their neonatal stay. Grade 1/Germinal matrix haemorrhage appears on the CRUS as an echoreflective area in the caudo thalamic groove, while higher grades of IVH appearances are marked as echoreflective opacity inside the ventricular cavity (56).

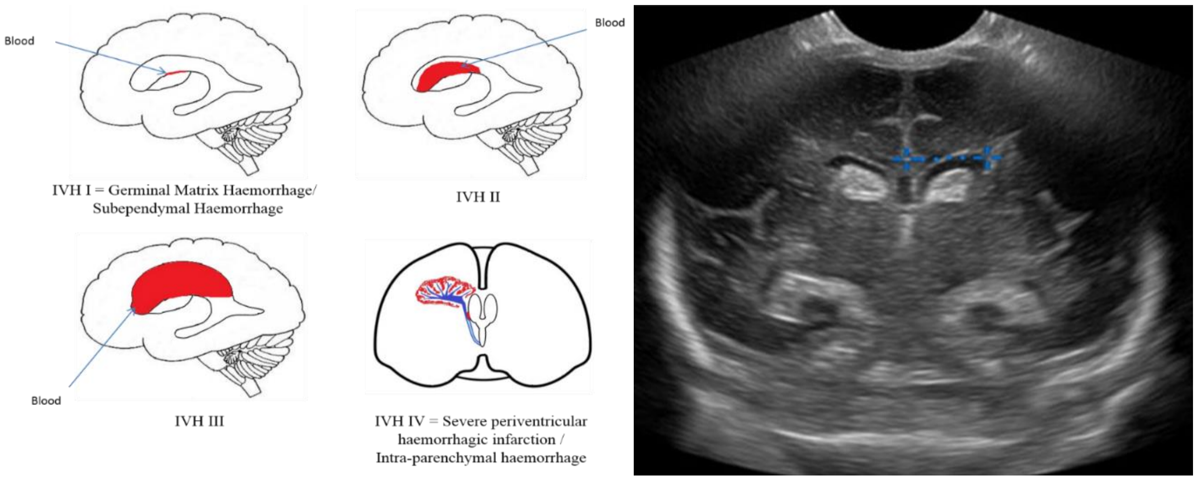


Figure 1-6 Intraventricular haemorrhages at four different grades of severity (IVH I-IV) and CRUS image of Bilateral Intraventricular haemorrhage from 29 weeks infants on day one following birth. CRUS image is reprinted from Mazmanyan et al (57).

Prognosis depends on the grade of the IVH, with contradictory reports regarding low grade IVH (grade 1 or 2) in terms of adverse outcome. Payne et al. reported that, from a cohort of 270 infants with low grade haemorrhage and 1021 infants with no haemorrhage, no difference in neurodevelopmental outcome was evident between the groups (58). This is also supported by a recent study from Reubsaet et al. reporting that low grade IVH very preterm infants have similar outcome to control infants without evidence of brain injury (59). Other studies however, have reported an association with mild to severe neurodevelopmental outcomes. Bolisetty et al. described how adverse neurodevelopmental outcome was evident in infants with grade 1 or 2 IVH, including neurosensory impairment, developmental delay, CP and deafness (60). It has been suggested that the glial precursor cells of the germinal matrix are damaged even during low grade VH. The development of oligodendrocytes and astrocytes is consequently interrupted, leading to disturbance of migration necessary for organisation of the cortex (61). Severe IVH (grades III and IV) is more likely to be associated with adverse neurodevelopmental outcome. It has been reported that 71% of infants with grade IV IVH/ Intraparenchymal haemorrhage (IPH) developed CP, while infants with grade I, II and III IVH developed CP 7.2%, 17.3% and 23.1% of the time respectively (62).

1.4.2.2. Periventricular Leucomalacia

Periventricular leucomalacia (PVL) is ischemic white matter (WM) damage in the immature brain. PVL can be classified as either cystic or non-cystic. Cystic PVL can develop when decay occurs at the site of damage and becomes cystic, while non-cystic PVL occurs when the small necrotic lesions do not cavitate, leaving evidence of diffuse WM gliosis. The cystic PVL that is visible on CRUS represents only the tip of the iceberg (63). Non-cystic is more common and is best seen on MRI as it can reveal areas of white and grey matter atrophy (64), while punctate WM lesions can also be present (27). Expert users can also identify non-cystic PVL via CRUS due to the ability to detect increased abnormal echogenicity of the WM. Due to advances in neonatal care, including antenatal steroids, surfactant and new ventilation strategies, cystic PVL is less frequent, affecting only 3 – 5% of infants below 32 weeks (56, 65-68). Although the incidence of cystic PVL has reduced recently, it remains a high risk brain injury that leads to CP and other neurodevelopmental impairments (69).

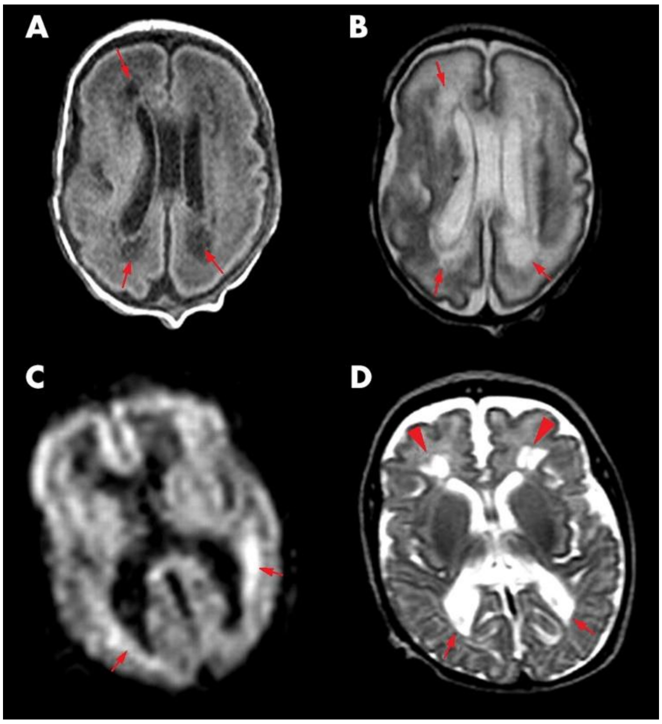


Figure 1-7 MRI images of PVL in an infant at 28 weeks GA. Arrows show areas of cystic lesions and areas of restricted diffusion around the lateral ventricles (70).

WM damage can become widespread, however it typically involves the periventricular WM regions. Inadequate perfusion via the long penetrating arteries, which supply blood to the deep WM, causes decreased blood flow to the WM, which leads to death and degeneration of tissue (71, 72). Necrosis of the WM leads to generation of cysts in the periventricular region in the brain, next to the lateral ventricles. Cerebrovascular blood flow in preterm infants is poorly controlled and can lead to decreased blood flow to the WM (71). The lack of anastomoses in the immature brain and failure to autoregulate the 'pressure-passive circulation' means that if the blood pressure decreases, the CBF will decrease and cerebral perfusion is affected, leaving the brain in danger of ischemic injury (73). The most vulnerable area for damage is the border zones between the deep penetrating arteries and the end zones of short penetrating arteries, also known as the 'watershed zone' (74, 75). The WM in this region contains nerve fibres that travel from the brain to the muscles. PVL is associated with ischaemia, maturational dependent vulnerability of the oligodendrocytes, or cytokine-induced damage following perinatal infection (71, 72). The cerebral white matter between 24 – 43 weeks GA is predominantly populated by developing oligodendrocytes. Pre-oligodendrocytes are vulnerable to excitotoxic, oxidative and inflammatory injury, with release of pro-inflammatory cytokines injuring the immature brain (76). It is believed that hypocarbia and hypotension increase the risk of PVL (77, 78), while it is also reported that perinatal and fetal infections have an association with WM damage. The cytokines interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α), that are produced by the microglia and astrocytes, can cause cytotoxic damage to the oligodendrocytes (79, 80).

A PVL classification was devised by De Vries (81): Grade I PVL is a prolonged periventricular flare apparent for 7 days or more. Grade II PVL is presence of echodense areas evolving into lateralized fronto-parietal cysts. Grade III PVL is an extensive periventricular cystic lesion involving occipital and fronto-parietal WM. Grade IV PVL involves regions of extensive sub-cortical cystic lesions in the deep WM (81). The development of PVL often develops late and may not be detectable for 4 – 8 weeks of life, therefore a CRUS in the first few weeks of life might not be useful (82).

Cognitive impairment and general motor delay is a common diagnosis in premature infants, while the development of CP is also possible. In a large study (EPIPAGE) of preterm infants between 22 – 32 weeks GA, 75% of infants with bilateral cystic PVL developed CP (83).

1.4.2.3. Infection

Neonatal infection is acquired during prenatal development or the neonatal period. Sepsis is a condition caused by the body's response to an infection and can cause long-term neurodevelopmental impairment and mortality (84). Preterm infants have a higher susceptibility to infection due to an under developed immune system (84). It is reported that 10 - 25% of preterm infants below 32 GA develop sepsis in the first week of life (85). Sepsis is categorised as early onset sepsis (EOS) and late onset sepsis (LOS). EOS presents in the first 72 hours of life, while LOS is present after the first 72 hours.

Early Onset Sepsis

EOS has an incidence rate of 1 – 2 per 1000 live newborn births and a mortality rate of 16% in preterm infants (86), however this depends greatly on the infective organism. The infective microorganisms can appear from transplacental infection or an ascending infection from the cervix. The most common microorganisms found to cause EOS are Group B Streptococcus (GBS) and Escherichia coli (E.coli). GBS is a commensal organism of the female genital tract (87, 88). Intrapartum maternal prophylaxis has been introduced for GBS, which has reduced the incidence rate by 80%, however, GBS remains a common cause of EOS (89). E.coli is a gram negative bacterium, which is the most common pathogen in preterm sepsis (90). Combined, GBS and E.coli accounts for approximately 70% of preterm infections. A study showed that 81% of infants with EOS due to E.coli, were in fact preterm infants (89).

Late Onset Sepsis

Twenty-five – 30% of very low birth weight (VLBW) preterm infants (86) and 38% of extremely low birth weight (ELBW) preterm infants (91) develop LOS. LOS is a nosocomial hospital acquired infection, rather than maternal or birth related factors. The most common microorganism causing LOS is Coagulase-negative staphylococci (CONS). CONS sepsis generally occurs from invasive procedures. It is the most common pathogen of gram positive infections, with an infection incidence of 68% (90). Staphylococcus aureus is another harmful gram positive bacterium that causes LOS, with an incidence rate of 8% (90).

1.4.2.4. Necrotizing Enterocolitis

Necrotizing enterocolitis (NEC) is a complication occurring in the preterm infant, characterised by infection, inflammation and necrosis of the intestinal tissue. This is a serious condition that can lead to disability and neonatal mortality. It occurs in approximately 5% of very preterm infants, and approximately 10% of all extremely preterm infants (92, 93). The pathogenesis is multifactorial, however it is believed to be mostly associated with three underlying factors, namely bacterial infection, intestinal hypoxia and feeding (94, 95).

A method of clinical staging was proposed by Bells et al., and modified by Lee and Polin (96, 97). This comprised of Stage IA and B of suspected NEC, stage IIA and B of proven NEC, and stage IIIA and B of advanced NEC (97). The clinical presentation of infants with NEC varies. Gastrointestinal non-specific symptoms such as apnoeas and lethargy can be identified, but more specific infants show abdominal signs of abdominal distension and bloody diarrhoea (27). Its appearance can be investigated by x-ray, where dilated loops and pneumatosis can be identified.

Stopping feeds and relieving distension, antibiotics, fluids and nutritional treatments are the initial approach, however when the disease is advanced, at Bell stage IIIA, surgery may be required (97). Mortality is a possible outcome, with an overall incidence rate greater than 10% (92). Moreover, infants who recover from NEC are still at risk of future complications.

Infants who are diagnosed with NEC are at significant risk for undernutrition, because of decreased absorption of nutrients due to mucosal injury (98). Brain development is dependent on nutrition and deficiencies can lead to lack of cortical growth, particularly at this critical stage of brain development. Complications following NEC include gastrointestinal sequelae such as intestinal stricture, reported in 20%, (99) and short bowel syndrome, reported in approximately 25% (100, 101). Furthermore, adverse neurodevelopmental outcomes are likely following NEC, namely WMI, cognitive and motor impairment (98).

1.4.2.5. Chronic Lung Disease

CLD, often known as bronchopulmonary dysplasia (BPD), is a respiratory condition caused by tissue damage in the lungs (102). This commonly occurs in preterm infants, who were initially born with RDS, where the undeveloped lungs were unable to provide the body with enough oxygen (27). CLD is extremely common in preterm infants, especially extremely premature infants, with a recent study reporting that moderate or severe CLD was diagnosed in 81.8% of infants 22-23 week GA, 79.4% of 24 week GA and 60.7% of infants at 25 week GA (103).

Factors that increase the likelihood of developing CLD include prematurity and the resulting RDS. In addition, oxygen toxicity, pulmonary volutrauma and patent duct arteriosus have also been linked to the condition (27). Oxygen toxicity may occur due to the large amount of cytotoxic oxygen free radicals and low amount of antioxidants in the lungs, making the tissue vulnerable to damage (27). Volutrauma results from ventilation, when high tidal volume leads to over-distention of the lungs (104). The association of PDA with CLD is fluid overload, potentially deteriorating the function of the lung (104).

It is believed that CLD can lead to cognitive and motor impairment, brain damage and CP (105). Episodes of hyperoxia are reported to be a potential factor in these complications (106). A recent animal study investigated this theory by exposing rats to repeated hyperoxia (95% O_2) and discovered that this resulted in an increased mean linear intercept in the lungs, which is a widespread parameter evaluating lung structure by measuring morphometric lung changes. This increase had an association with decreased volume brain structures and generally the size of the whole brain surface. Specifically, the anterior and posterior areas were affected mostly by repeated hyperoxia (107). Further research is needed, however it does appear that frequent episodes of hyperoxia is damaging to the brain.

Infants born less than 32 weeks who have had treatment with 21% oxygen (or more) lasting for at least 28 days will be reviewed by a clinician at 36 weeks post-menstrual age (PMA) or at discharge to confirm the diagnosis of CLD. The severity of CLD for infants below 32 weeks is included below:-

- Mild CLD - Required 28 days oxygen treatment, but breathing room air by 36 weeks PMA / at discharge

- Moderate CLD - Require <30% oxygen at 36 weeks PMA / at discharge
- Severe CLD - Require >30% oxygen at 36 weeks PMA / at discharge (104)

1.4.2.6. Retinopathy of Prematurity

ROP is a severe condition of the eye, where abnormal development of blood vessels occurs in the retina (108). ROP is one of the main causes of childhood blindness and is associated with non-visual neurodisability at 5 years of age (109). The incidence of ROP in extremely preterm infants ranges from 33 – 38%, with 35% of all ROP being severe (110, 111). The retinal blood vessels begin to develop during the fourth month of gestation, therefore premature infants are born with under developed, non-vascularized eyes (112). ROP occurs when hyperoxia of the extrauterine environment prevents the normal retinal vascular growth. This can lead to loss of some already developed vessels due to tissue hypoxia (27). It can then develop further when vessel proliferation occurs, otherwise known as 'retinal neovascularisation'. Over time, the growth of vessels can produce a scar, causing retinal detachment from the epithelium, resulting in blindness (113).

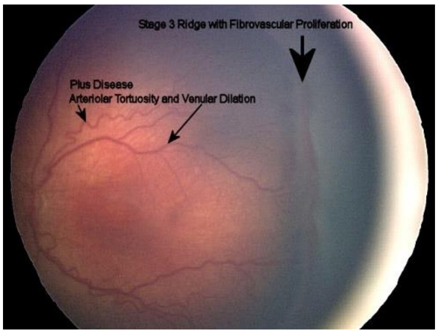


Figure 1-8 Pathology of Retinopathy of Prematurity Reprinted from Wilson & Fielder 'Retinopathy of prematurity' (114).

Screening involves repeated dilated eye examinations on infants born below 30 weeks GA or BW of <1500g (108). Following examinations, ROP abnormalities can be categorised by the International Classification of Retinopathy of Prematurity (115):

- Stage 1 – Demarcation Line: line between the vascularised and non-vascularised areas of the retina.
- Stage 2 – Ridge: the line increases in depth and height
- Stage 3 – Extraretinal Fibrovascular Proliferation: vessels extend into the vitreous
- Stage 4 – Partial Retinal Detachment
    - A – Extrafoveal
    - B – Fovea
- Stage 5 – Total Retinal Detachment

Both cryotherapy and laser surgery are possible treatment options for ROP. Both strategies aim to prevent the growth of abnormal blood vessels. Cryotherapy freezes areas of the retina that have not developed blood vessels, while laser treatment uses laser beams to scar the retina.

1.4.3. Treatment of Preterm Infants

1.4.3.1. Medication

Preterm infants are often treated with medication during their stay in the NICU. The National Institute for Health and Care Excellence (NICE) guidelines recommend the administration of antibiotics such as benzylpenicillin and gentamicin to treat or prevent infection (116). Additionally, antenatal corticosteroids are used to aid lung function and development by preparing the lungs for respiration. Antenatal corticosteroids can be given before birth to reduce the risk of developing RDS, while postnatal steroids are used to treat the already apparent RDS and manage the severity of the condition (117). Surfactant is also administered to replace the surfactant deficiency that is naturally present in developed lungs (118). Inability or difficulty in breathing can cause apnoeic episodes and the infant can be treated with the central nervous system stimulant, Caffeine Citrate (119). Opioids are commonly used in the NICU, acting as a pain relief in situations such as NEC or for procedures such as intubation (120). Commonly used opioids include morphine and fentanyl.

1.4.3.2. Anti-epileptic drugs (AEDs)

Preterm infants in the NICU present a vast repertoire of general movements that may be mistaken for clinical seizures and treated erroneously. A neonatal seizure is the result of excessive synchronous electrical discharge from depolarisation of neurons in the brain, which can be observed using EEG (14). Anti-epileptic drugs (AEDs) can be prescribed, with or without EEG monitoring, in an attempt to prevent or stop seizures from occurring. In the NICU, infants might have clinical and electrographic seizures. Electrographic seizures can only be detected by an EEG as no clinical manifestations are present.

Seizures disrupt the electrical bursting patterns of the brain that are essential for neuronal survival and connectivity and which consequently contribute to longer term neurodisability (121-123). Therefore, reliably diagnosing seizures is important. It is equally important, however, to avoid treating infants unnecessarily, as AEDs can have neurotoxic effects (124-126). Caution has been advised for the treatment of preterm infants, as early AED exposure has shown reduced brain mass, due to apoptotic neurodegeneration in the developing brain, in addition to an association with adverse cognitive outcome (127). Preterm infants may be at higher risk of AED neurotoxicity due to a lack of maternal β-Estradiol, which usually has an inhibitory effect on AED neurotoxicity (127). AED treatment of seizures in preterm infants can lead to hypotension and sedation (128).

In full term infants, phenobarbitone is generally used as the first line drug of choice, followed by phenytoin and benzodiazepines (129, 130). Appropriate AED treatment for preterm infants is less clear, however one study reported no apparent difference between term and preterm infants, in terms of medication choice. This study showed that phenobarbitone was the most popular choice (72.2%), with phenytoin acting as the second-line agent (40.6%) (130). Phenobarbitone is a GABA-mediated inhibitory neurotransmitter, which delays the GABA-mediated Cl^-

channels from closing and consequently prevents synaptic excitability (129). Phenytoin acts on voltage-operated ion channels, or more specifically, via membrane-potential-dependent blockade of Na+ channels (131). Benzodiazepines such as clonazepam, lorazepam, and midazolam are common third line AED choices and are also GABA-mediated inhibitory neurotransmitters. They can also act on specific subunits of $GABA_A$ channels to enhance the action of GABA (132). In addition, some seizures can be very difficult to control, and consequently other AEDs can be introduced. A recent study showed how only 26% of preterm infants <28 weeks GA responded to Levetiracetam (133). Other AEDs such as carbamazepine, chloral hydrate, lidocaine, paraldehyde, sodium valproate (Epilim), topiramate, vigabatrin and bumetanide have been used to treat neonatal seizures.

Although phenobarbitone and phenytoin are used as first line seizure treatment, research shows that their ability to effectively treat seizures ranges from 30 – 50% (128, 134-136). A possible reason for this is that the neonatal brain is still developing. As a result, GABA receptors are also under-developed (129). Animal studies and in vitro studies have been central to the 'excitatory' GABA hypothesis, which has been linked to the susceptibility of the term and premature brain to seizures and hyperexcitability. It is suggested that GABA primarily has an excitatory role during prematurity, which switches to a mature inhibitory role during the second post-natal week (137). The core of this theory revolves around the differential expression of the NKCC1 and KCC2 transporters in infants. High amount of NKCC1 expression occurs during early development, which leads to net efflux of negative current, causing cell depolarisation. The opposite effect occurs during the post-natal period, when KCC2 expression is increased, causing hyperpolarisation of the neuron and ultimately effective inhibition (137, 138). With this theory in mind, the expectance would be for anticonvulsants such as phenobarbitone to increase seizures in preterm infants, rather than to decrease seizure activity. However, this has not been clinically reported and phenobarbitone continues to be useful in many infants. Further research is clearly needed in this area as phenobarbitone continues to be the first line drug of choice for all neonatal seizures.

1.4.4. Neuro - imaging & Monitoring the Preterm Brain

The premature brain is vulnerable to injury due to multiple factors such as a fragile network of blood vessels and immature oligodendrocytes. The two main imaging modalities used clinically in preterm infants are CRUS and magnetic resonance imaging (MRI). Other neuro-monitoring tools are used to observe the physiological activity of the brain, such as near-infrared spectroscopy (NIRS), electroencephalography (EEG) and amplitude-integrated EEG (aEEG).

1.4.4.1. Cranial Ultrasound (CRUS)

CRUS was introduced to the NICU in the 1970-80s and has a role in identifying IVH (66). CRUS is the preferred neuroimaging choice for diagnosis of IVH and ventricular dilation in preterm infants. Additionally, it is also convenient as it is mobile, non-invasive and easy to use in the NICU. It takes advantage of the fontanelles, by allowing reflected ultrasound waves to travel through the aperture, to produce the images (14). This provides access to images of the central regions of the brain, ventricles and periventricular areas. CRUS is a well-regarded tool with a high accuracy in identifying IVH (139-141). A study by Franckx el at, investigated early and late CRUS, MRI, SSEP and EEG in premature infants <32 weeks GA, and neurodevelopment outcome at 2 years of age. Performance of these diagnostic tests were inconclusive. Normal EEG did not change the probability of adverse outcome, with negative likelihood rations of between 0.49 and 0.98, however the best predictor of outcome was the early CRUS (142). This study investigated infants less than 32 weeks GA, however the EEG was not performed until 33 – 34 weeks. Furthermore, there is no indication about how long the EEG monitoring lasted for, therefore a possible reason for the poor performance could be lack of continuous data during the early postnatal period.

Limitations are also acknowledged, one being that lesions in the peripheral brain areas might be harder to examine and diagnose, such as smaller punctate lesions and posterior fossa lesions (143-145). Infants <30 weeks GA at CUMH undergo routine CRUS screening in the first week (day 1-3 if possible), a repeat scan before two weeks of age, then another pre-discharge. Multiple scans might be performed on the extremely preterm infants.

1.4.4.2. Magnetic resonance imaging

Magnetic resonance imaging uses a magnetic field and hydrogen ions to produce high resolution images of body parts, such as the brain. Entering the strong magnetic field causes the hydrogen protons of the body to align in a uniform direction (146). When a radio frequency wave is transmitted through the brain, the protons become excited and spin out of alignment. Turning off the radiofrequency waves will cause the protons to realign back to their previous state. This realignment, or relaxation of the protons emits a signal which is used by the imaging computer to create images. These energy signals return at different strengths and times allowing the different structures and tissues to be identified. MRI is regarded as the best method of assessing normality and some abnormalities such as cerebellar haemorrhage (147), however it does not measure function.

A review of the predictive ability of neuro MRI in preterm infants for neurodevelopmental outcome has shown that that the role of advanced MRI techniques is improving, however further studies are needed (148, 149). Studies have used techniques including volumetric MRI (vMRI) (150-152), diffusion tensor imaging and diffusion MRI (dMRI) (153, 154), magnetic resonance spectroscopy (MRS) (155), and resting-state functional connectivity MRI (fcMRI) (156, 157) to identify early prognostic biomarkers such as subtle structural or functional connectivity and metabolic abnormalities to improve predication accuracy. Nonetheless, a combination of sequential CRUS and MRI recordings can be used to increase the prognostic effectiveness and sensitivity of preterm brain injury diagnosis (158). A study by Hintz et al. reported how abnormalities such as white matter abnormality or cerebellar lesions from a late CRUS and MRI at near term were associated with adverse outcomes between 18 and 22 months (159), while the same associations were found in another study by Anderson et al., when assessed at 7 years of age (160). MRI does have significant shortcomings for the assessment of preterm infants in the NICU environment, such as its accessibility, transfer, expense and safety (158). It is very is challenging to perform early MRI, as preterm infants frequently require respiratory support. Preterm infants need to be well enough to leave the NICU and go to the Medical Imaging Department, whereas in comparison, the CRUS or an EEG can be brought to the patient bedside.

1.4.4.3. Near infrared Spectroscopy

Near infrared Spectroscopy is a non-invasive tool that uses infrared light (with wavelengths between 650 and 950 nm) to measure concentration changes in cerebral haemoglobin to determine regional cerebral tissue oxygen saturation (161). The NIRS probe is positioned on the scalp to enable measurement of these changes (162). Haemoglobin can be oxygenated or deoxygenated, and when the light is applied, it is absorbed by haemoglobin, with the oxygenation status affecting the amount of absorption undertaken (163). The device is portable, painless and the results are readily available on the screen, while no ionising radiations are introduced (163). Limitations are apparent however, such as its precision, its dependency towards the observers' experience due to difficulties interpreting the values, and also the possibility of leaving a burn on the scalp (163). Numerous studies involving NIRS are ongoing, including the SafeBoosC randomized trial, which investigates its role in preterm infants' cerebral oxygenation (164). A study investigating the relation between NIRS and EEG activity in preterm infants discovered that higher values of fractional tissue oxygen extraction from the NIRS signals were related to higher values of EEG amplitude (165).

1.5. Electroencephalography (EEG)

Electroencephalography (EEG), records the electrical activity of the brain. As EEG is the core of this thesis, its history, recording principles and the maturational aspects of its appearance in preterm neonates will be described in more detail below.

1.5.1. History of EEG

The first human EEG was performed by German psychiatrist Hans Berger (Figure 1-9) in 1929, which revolutionised the world of neurology and psychiatry. Berger's inspiration was Englishman Dr Richard Caton, who in 1929 first used a galvanometer on monkeys and rabbits to determine the existence of electrical current from the grey matter of the brain (166). Berger began his research by using an Einthoven string galvanometer. He later used the Edelmann models, before producing ground-breaking work with the Siemens double coil galvanometer. This was the machine that recorded the first ever human EEG with non-polarisable pad electrodes. One was applied over the frontal and occipital region to produce a single bipolar channel recording of 3 minutes on to photographic paper (167). Berger identified the alpha and beta waveforms and proposed the term "electroencephalogram" (168).

Figure 1-9 Picture of Hans Berger reprinted from Niedermeyer E, da Silva FL, Electroencephalography: basic principles, clinical applications, and related fields (167).

In 1934, Lord Edgar Douglas Adrian, replicated Berger's work by applying electrodes on himself and producing alpha waves when his eyes were closed. He increased the awareness of Berger's work and confirmed its credibility (167).

EEG devices have developed over the years, with historic paper based machines now superseded by modern digital devices sampling the analogue signal to create digitised data. The advantage of digitisation is that it is now possible to change settings, re-montage, employ various signal processing techniques and annotate this data retrospectively, which was previously impossible once the waveforms were printed on paper. With large data storage now built into modern devices or available on servers, monitoring brain function can be performed over long periods, enabling clinicians to monitor ongoing seizures and titrate AEDs appropriately and assess the development of the disease process in acutely ill infants. An additional advantage of digitisation is the ability to create digital copies allowing sharing of data amongst medical professionals and greater robustness to data degradation loss thus improving data security, and also negating the requirement to store abundant volumes of paper recordings.

1.5.2. Physiological principles of EEG

Electroencephalography is a visual trace of voltage difference between two cerebral areas over time. Recording electrodes positioned on the scalp capture signals generated by cortical neurons due to the conductive properties of the tissue. The electrical activities are produced by extracellular current flow associated with summated excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs). The EEG is derived primarily from these generation of summated (EPSPs and IPSPs), rather than the presynaptic action potentials. The polarity of an EEG wave, recorded from the scalp, is dependent on the net charge of the interstitial fluid at the most superficial region of the cortex. This electrical charge is conducted through the meninges, scalp and skin to the electrode. The net charge within a specific region of the cortex is dependent on the net charge of EPSs and IPSPs arriving at the dendritic post synaptic membrane causing a net charge at the superficial interstitial fluid. Influx of Na+ into the dendrite, due to an excitatory action potential arriving from the thalamus, increases the internal positive charge, leaving the interstitial fluid around the synapse to have a negative charge. The excitatory Na+ current runs up the axon and exits to the interstitial fluid most superficial to the cortical surface leaving a net external positive charge. This is conducted to the electrode and displayed as a downward deflection. The opposite occurs when an excitatory axon synapses onto the distal portion of the dendrite, leading to an upward deflection. The principle for excitatory inputs is illustrated in figure 1-10. (169).

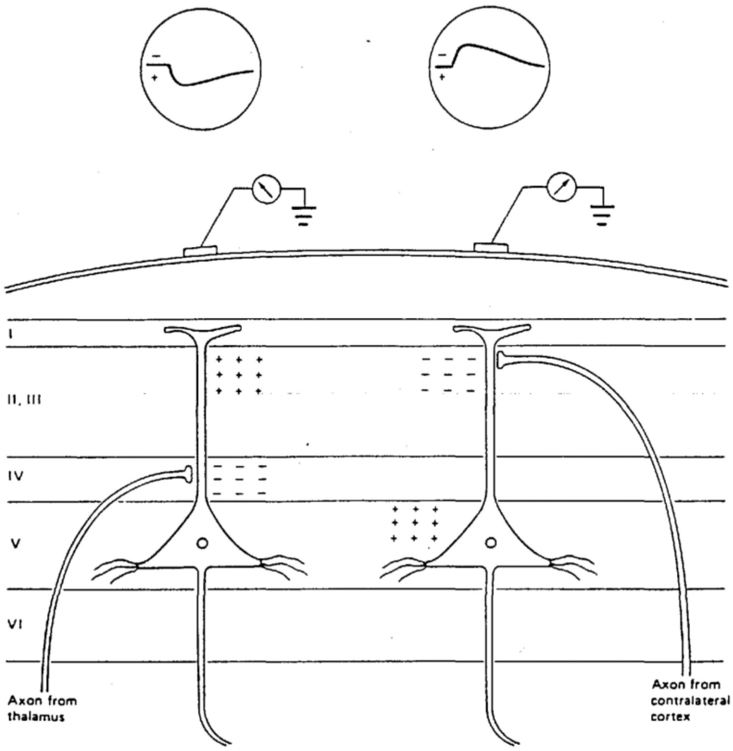


Figure 1-10 The origin of EEG potentials, where a downward EEG deflection occurs from a thalamic input to the proximal dendrite and an upward deflection from a thalamic input to the distal dendrite. reprinted from Olejniczak P. 'Neurophysiologic basis of EEG' (170).

The internationally standardized 10-20 system was developed to ensure that EEG recordings were performed accurately universally (171). The guiding principle is that if all recording centres adhere to this application system, recordings made at different centres can be compared with the confidence that they are recording from the same brain regions with similar parameters. In the adult version of this system, there are 21 electrode positions on the scalp surface (Figure 1-11). Application begins by measuring the head, by locating the nasion (the indentation between the forehead and the nose) and inion (the bony protuberance in the middle of the back of the head) positions. The preauricular points of the ears are also located, then the skull perimeters can be measured medially and transversely. The perimeters are divided into 10% and 20% intervals to identify the electrode positions (172). The abbreviations refer to the electrode positions on the scalp, with the letter referring to the region (Fp, Frontal-polar; F, Frontal; C, Central; T, Temporal; P, Parietal; O, Occipital and A, Auricular), number referring to the hemisphere (odd numbers=left, even numbers = right) and 'z' referring to the midline. The ground electrode can be placed anywhere on the head, however behind the ear on the mastoid bone is often used, which is referred to as the auricular electrode, while a reference electrode also needs to be applied which is usually placed on the midline.

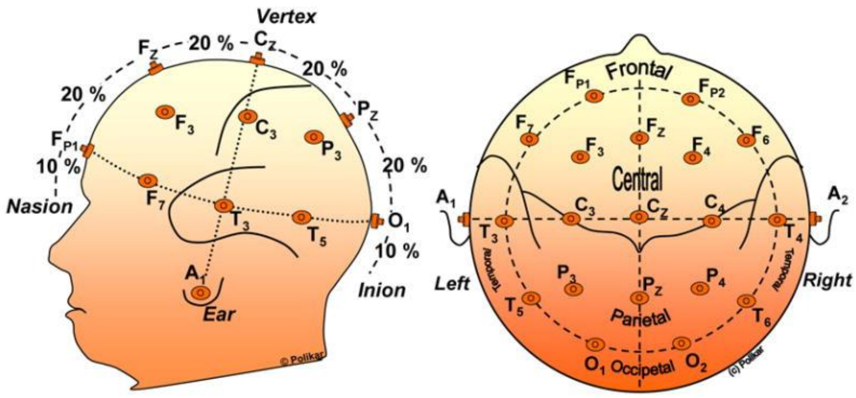


Figure 1-11 The international 10-20 measuring system for EEG placement, standardised by the American Electroencephalography Society seen from the sagittal and axial perspective; reprinted from Polikar R,* et al. *Comparative multiresolution wavelet analysis of ERP spectral bands using an ensemble of classifiers approach for early diagnosis of Alzheimer's disease. (172).

EEG signal acquisition involves detecting electrical brain potentials in microvolts (µV) from scalp electrodes, and converting them from analogue to digital values. The continuous differential analogue signal of the differential electrical potential between two electrodes is amplified before being converted to digital signal, via the analogue to digital converter (ADC). Once amplified, the signal is filtered with a bandwidth of 0.5 to 70Hz. This removes slower frequency to avoid a 'drift' of the EEG trace, while the faster frequencies are removed to prevent aliasing. Once filtered, the analogue signal is converted to digital signal. This involves sampling the signal at a high frequency such as 250Hz (or similar) to create a finite set of samples, 250 in this case, per second. Following this, the data can be stored on a hard drive for future revision or it can be visually displayed on a screen/monitor.

Using the international 10-20 system for electrode application on infants can be challenging due to the smaller size of the head. The system can be modified to accommodate the head size by only applying 11 electrodes. The positions typically used are F3, F4, C3, C4, T3, T4, O1, O2, Ground (A1) and Reference (Fz).

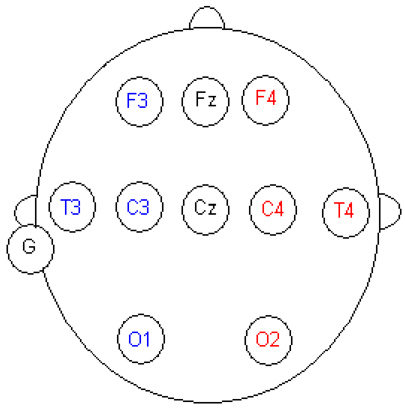


Figure 1-12 International 10/20 system modified as used in neonates.

EEG traces can be visually analysed either during the real time recording and/or post acquisition. To ensure a comprehensive review is achieved, the EEG should be viewed from different perspectives, and not only the way in which the data was recorded. The method for achieving this is to view the EEG in a combination of montages. A montage is a pattern of how the connecting electrodes are represented. Changing the montage enables the opportunity to view electrical activity at different electrode positions. The EEG is initially recorded in a referential montage. This is when every active electrode is referred to a common reference electrode, Fz in our case. From this derivation it is possible to change to other montages, such as the average reference montage or bipolar montages. An average reference montage is when each active electrode is referred to the averaged outputs of all the electrodes. Bipolar montages are adjacent electrodes connected in longitudinal or transverse directions, with one electrode (lead 1) acting as the active electrode and the other (lead 2) acting as the inactive electrode. In a bipolar montage, a given electrode may be shared between electrode pairs representing an EEG channel but in different positions in the pair. For example, in Figure 1-1A C4 is lead 1 in the C4-02 channel but is lead 2 in the F4-C4 channel. In EEG, the convention is that a negative potential at an active electrode produces an upward deflection, however if the potential occurs at an inactive electrode a downward deflection is witnessed, where the potential at the inactive electrode is being subtracted from the active electrode. The consequence of shared electrodes in different positions in the electrode pair is that waveforms may become inverted on consecutive EEG channels, so called 'phase reversal'. This phenomenon can be useful to determine the origin of focal discharges or seizures on the EEG in a bipolar montage.

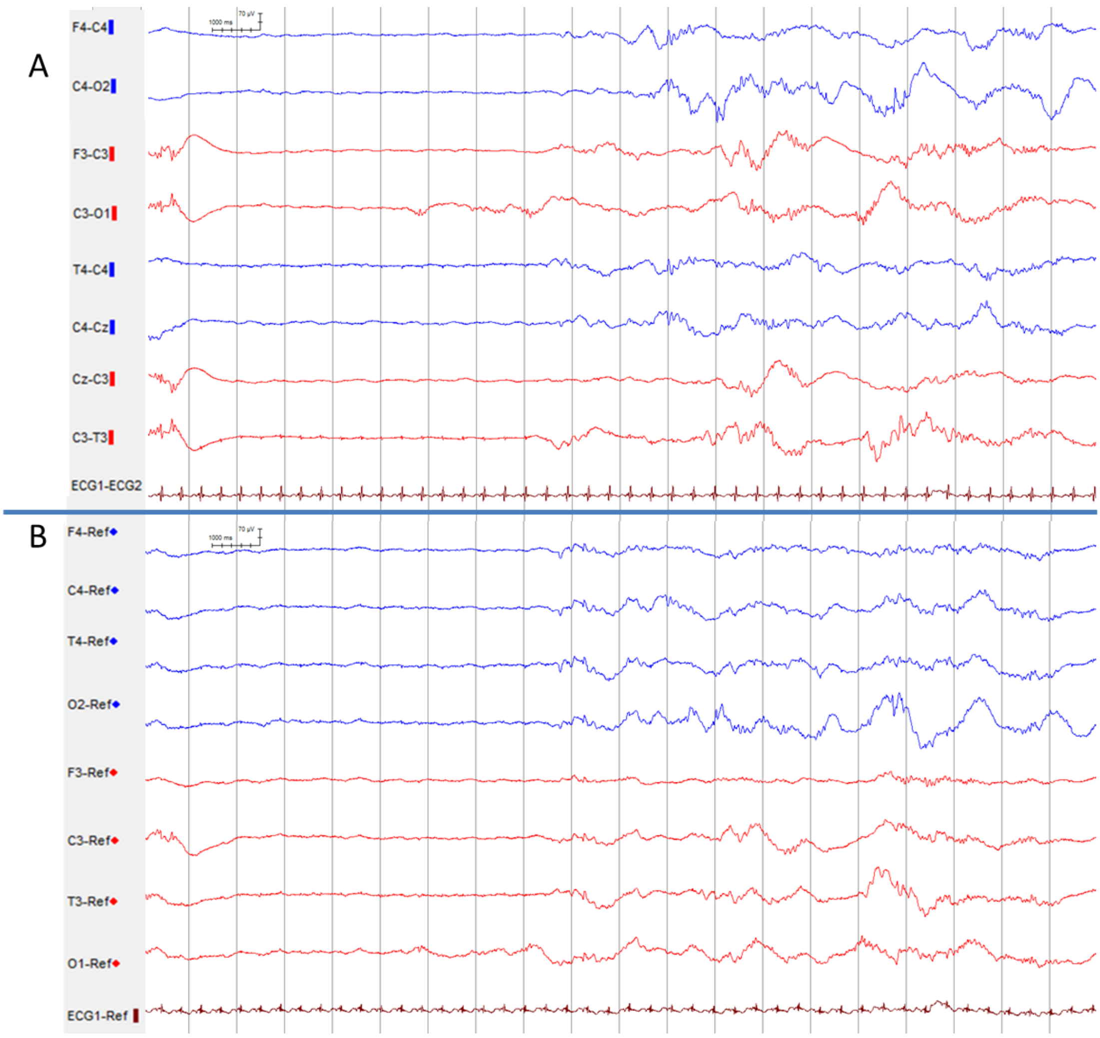


Figure 1-13 Example of a Bipolar and Referential montage from the same EEG recording: bipolar (A) and referential (B).

Montages are logical arrangements of electrode placements on the scalp for the display of the EEG activity. The montages display channels of waveforms of different amplitudes and frequencies. The frequencies of the EEG are usually categorised into frequency bands called delta (δ), theta (θ), alpha (α) and beta (β) (Figure 1-14). The delta band consists of the slower frequency waveforms of 0.5 – 3.5Hz, theta waveforms are slightly faster at 4 – 7.5Hz, alpha at 7.5Hz – 12.5Hz and the beta waveforms being the fastest at 12.5 – 30Hz.

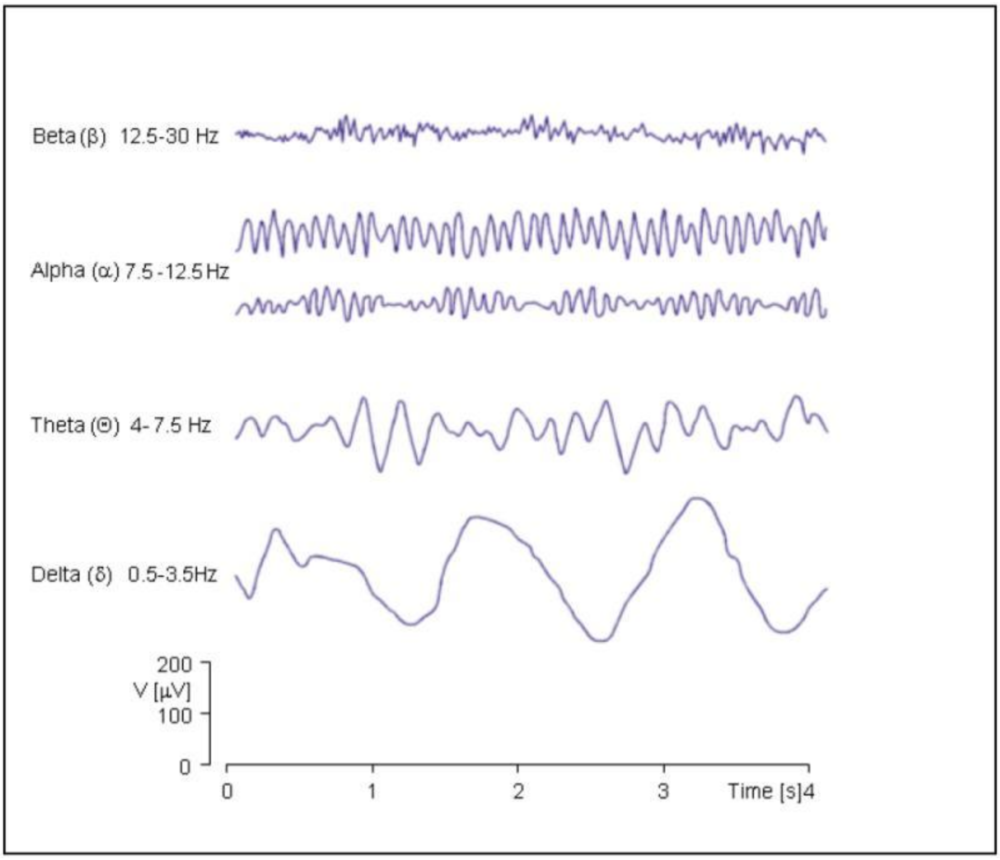


Figure 1-14 All EEG frequency bands derived from the raw EEG; reprinted from Tye, C **et al.** ***(173)***

1.6. Normal Preterm EEG

The EEG of preterm infants varies, depending on GA. As infants mature, specific waves appear/disappear, change in morphology, characteristics and organization. Figure 1 - 15 displays the features evident at different GAs. The evolving EEG reflects how the brain of a premature infant rapidly develops.

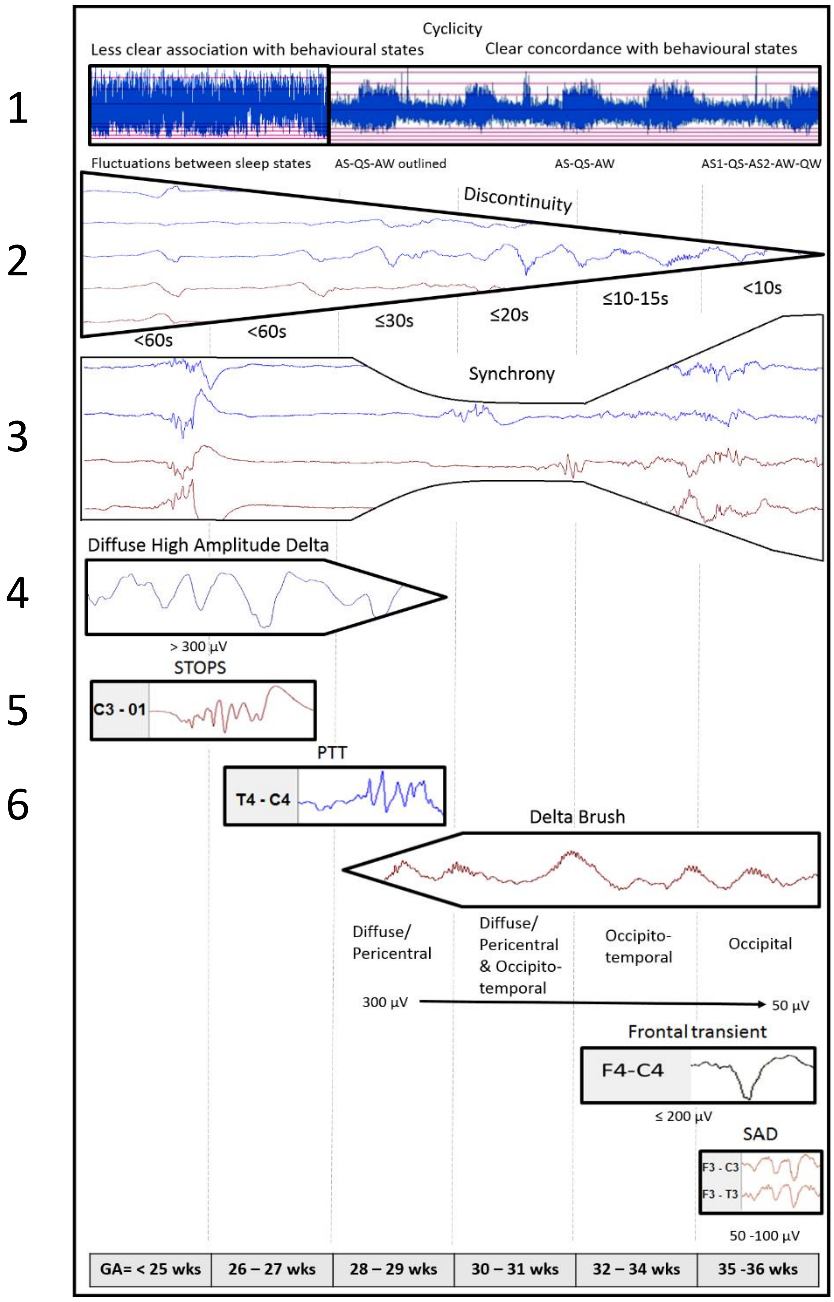


Figure 1-15 Maturation of preterm EEG features. Key: AS, active sleep; QS, quiet sleep; AW, active wakefulness; QW, quiet wakefulness; STOPS, Sharp theta on the occipitals of prematures; PTT, premature temporal theta; SAD, slow anterior dysrhythmia; GA, gestational age.

1. *Cyclicity:* Concordance between behavioural states and EEG states are only recognisable after 30 weeks GA (174). Previous studies described sleep-wake cycling as evident post 30 weeks GA while younger infants are in indeterminate sleep state (175). However, differentiation of sleep is detectable in younger infants when full polygraphic recordings of at least 1

hour is undertaken. The polygraphic features allowing for sleep identification are electrooculogram, electromyogram and respiratory monitoring (174, 176-179). Infants show alternating periods of continuous EEG activity with eye movements and discontinuous EEG activity without eye movements (174, 176). The duration of two successive periods has been reported to vary from 9 minutes 40 seconds to 55 minutes 20 seconds in 10 preterm infants between 24 weeks two days to 26 weeks four days GA (176). Curzi-Dascalova et al. previously reported a mean sleep cycle duration of 39.7 minutes in premature infants between 27 and 30 weeks GA (177), whilst Scher et al. reported a mean cycle duration of 68 minutes in a slightly broader range of GAs (25–30 weeks) (178).

Sleep-wake cycling relies on the maturation of interconnected neural networks located throughout the cortex, diencephalon and brainstem. The influence of deeper brain structures allows for cyclicity to be recognisable in the younger infants before proper thalamo-cortical connectivity has developed (179-181).

Sleep stages become more recognisable over time. At 35 weeks GA, the following sleep states and cyclicity should be expected (175):-

- Active wakefulness (AW) and quiet wakefulness (QW): characterised by continuous activity; in AW with mainly movement and muscular artefacts.
- Active sleep 1 (AS 1): high-amplitude continuous tracing, preceding quiet sleep (QS).
- Active sleep 2 (AS 2): continuous lower-amplitude tracing with more rapid activity, which follows QS.
- QS: discontinuous or semi-discontinuous tracing.

2. *Discontinuity:* The EEG pattern of a premature infant, is mainly characterized by discontinuous activity. This pattern is characterised by (active) bursts of high amplitude delta-theta activity intermixed with periods of (inactive) low

voltage activity, also known as IBI activity. With increasing GA over time, the duration of the IBI decreases, the burst duration increases, the overall amount of discontinuity decreases and the amount of continuity increases (182, 183). Therefore, an extremely preterm infant will have long IBI and short periods of bursts, but as the infant matures, the duration of IBI will shorten while the bursts will prolong (175). Tracé discontinu is a term used to explain these discontinuous EEG periods (175).

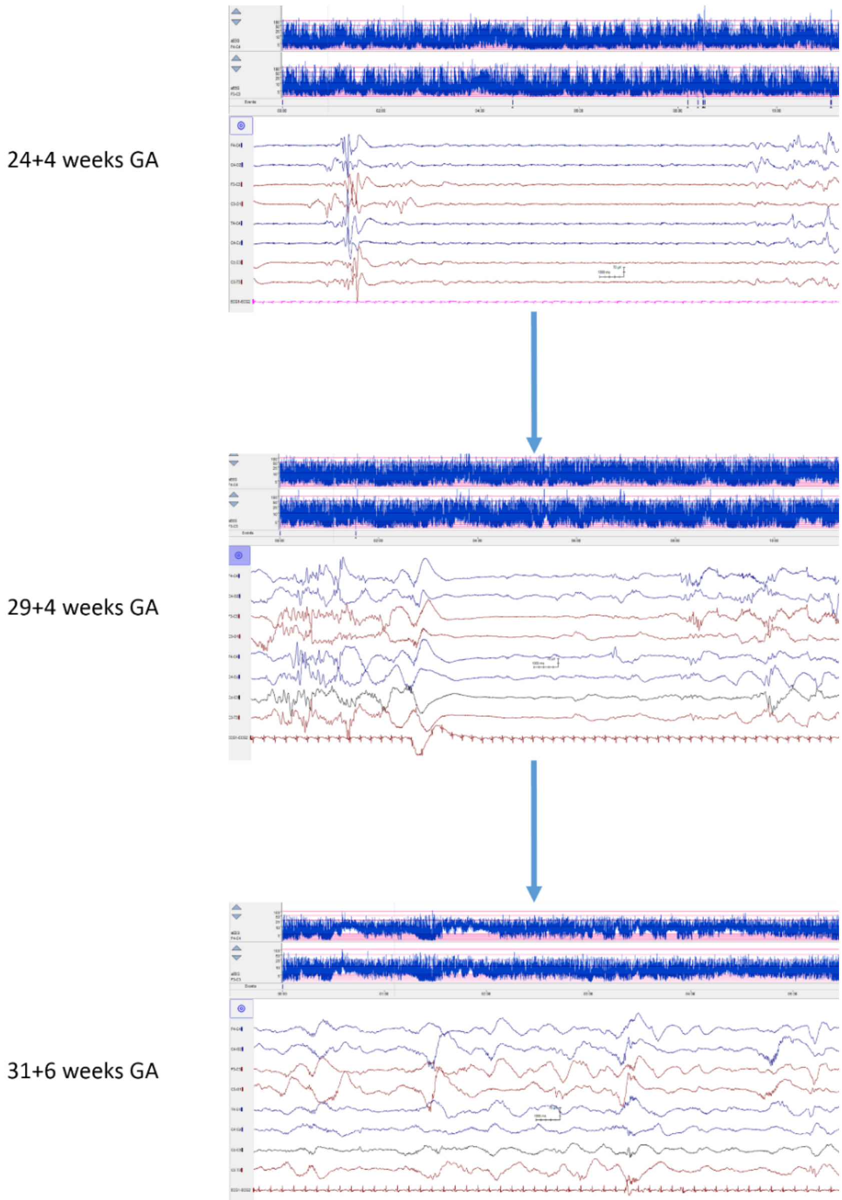


Figure 1-16 The EEG continuity change with increased GA. The initial discontinuous trace gradually becomes continuous with age.

Physiologically, discontinuity has been explained in animal studies which show that cortical structures present spontaneous, intermittent activity (121) that is a crucial endogenous driver for the development of brain connectivity before the cortical networks are modulated by the exogenous stimuli/sensory input (184, 185). Furthermore, early in development GABAergic transmission does not effectively inhibit the generation of the endogenous events (186, 187). Therefore, the electrical activity of early brain networks is characterized by 2 alternating modes of activity: the locally generated spontaneous activity transients (SATs) and the periods of relative silence between them (IBIs) (188). Continuous oscillations of different frequencies emerge while the normal inhibitory GABAergic transmission mature and SATs gradual disappear (189).

3. *Synchrony:* Presence of EEG synchrony is when all EEG features occur simultaneously in homologous areas over both hemispheres. Although interhemispheric synchrony has been shown to increase with increasing GA, synchronous bursts/IBI activity between the two hemispheres (paradoxical hypersynchrony) is present in preterm infants <30 weeks GA (175, 190, 191). Synchronous high-amplitude bursts of activity are evident as early as 24 weeks GA, with 88% of bursts being synchronous between 24 and 27 weeks, often in the occipital areas, and almost 100% between 28 and 29 weeks GA (174, 176).

   At 30 weeks GA, normal asynchronous physiological activity is witnessed. If this exceeds 50% of the discontinuous activity, it would be regarded as abnormal (190). This asynchrony continues, with gradual decrease in frequency, until 36 weeks GA before disappearing at term age (175).

   Synchrony is an important feature of EEG maturation, reflecting an immature cortex and the ongoing synaptogenesis of the corpus callosum (174, 190, 191).

4. *High amplitude delta:* This is a common feature, particularly evident <28 weeks GA. The amplitude exceeds >300 µV, while the frequency is as low as 0.3 - 1 Hz. Morphologically it is generally smooth and can appear as a mono- or diphasic wave. Superimposed faster activity can be evident over the centro-temporal regions. It is possible for the activity to appear unilaterally or bilaterally, however most notably over the bilateral occipital areas, and can continue up to <80 seconds (174-176).

5. *Sharp theta on the occipitals of prematures, or occipital sawtooth (STOPS):* These are regular rhythmical activities of 4 – 7 Hz, occurring over the occipital regions for a period of 0.5 – 3.5 seconds (192). The incidence is higher in the younger ages, with a peak at 25 weeks (193).

6. *Premature temporal theta (PTT):* These are runs of rhythmic theta activity of 4 – 6 Hz, usually bilateral, often asynchronous. They are usually slightly slower in frequency and higher in amplitude compare to STOPS. The incidence peak is at 29 – 31 weeks GA, but continues until 32 weeks GA in AS and until at 33 – 34 weeks GA in QS (175).

7. *Delta Brushes:* This is one of the most important and best understood features of preterm EEG. Delta brushes consist of fast rhythms (in the alpha-beta range) superimposed on a slow delta wave. These fast activities mainly appear on the ascendant slope of the slow wave (175, 194, 195). The peak incidence of delta brushes is between 32 and 35 weeks, however they have been reported in younger and older neonates, disappearing by 38 and 42 weeks (175, 195). Their amplitude decreases with maturation and their frequency becomes faster (174, 175, 194, 195). Topographically, delta brushes are initially diffuse, before becoming more predominant in central areas, in the temporo-occipital regions, then finally occipitally only at around 36 weeks (174, 175).

In infants less than 35 weeks GA, delta brushes can be elicited by different sensory inputs, in addition to being a spontaneous activity (195-200). Visual inputs evoke activity occipitally (197), auditory inputs evoke activity temporally (196), tactile stimuli to hand or foot evoke an activity in the lateral and medial regions of the contralateral central cortex respectively (199), while noxious stimuli in the heel can elicit delta brushes in the mid-temporal regions (198).

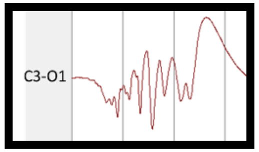
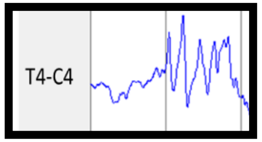
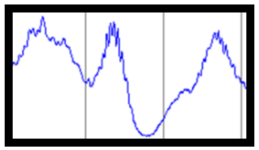
Delta brushes reflect the development of sensory functions and offer a marker to evaluate brain maturation (19). The somatosensory cortex develops vastly during the early stages of fetal brain development, with the gradual disappearance of delta brushes and gradual appearance of mature activity reflecting this process (197, 198, 201).

8. *Frontal transients:* These are sharp transients that usually appear synchronously at amplitudes up to 200µV (175). They are smooth, incomplete and asymmetrical in appearance, and are evident over the anterior regions. They can be seen at 33 – 35 weeks and with maturity, becomes more diphasic with a small negative deflection followed by a wider and higher amplitude positive deflection (175).

9. *Slow Anterior Dysrhythmia:* These are short sequences of delta waves appearing over the frontal areas. This feature appears in AS1 at around 36 weeks GA, reaching amplitudes of 50 – 100µV (175)

The duration of the IBI has been associated with the development of cortical folding of the brain, while sawtooth patterns such as STOPS and PTT are associated with the order of cortical folding, with the pattern present at 26 – 28 weeks over the occipital lobe, before appearing over the temporal lobe (183). As these features mature, continuity gradually becomes apparent. Table 1-3 specifies the background activity, features and states that are expected in the EEG of preterm infants, at specific age groups and how maturation gradually develops the EEG over time.

Articles by Andre et al., Vecchierini et al. and a book chapter by Boylan were used to devise this table (174, 175, 202).

CA (weeks)	Background	EEG features	Spatial & Temporal features	Behavioural state
24 – 25	Very discontinuous IBI : <60s Burst : >50µV at <60s Hypersynchronous	***STOPs*** : Sharp theta on the occipitals of prematures ***Occipital Sawtooth*** : Rhythmic, regular, occipital activities (4±7Hz at 0.5±3s) ***Mono / Diphasic waves*** : Smooth theta/alpha rhythms (>300µV at 0.3-1Hz) ***Theta waves*** : Sharp bursts of 200µV  STOPS	Frontal slow delta : Sparse Central slow delta : Monophasic, smooth with superimposed fast (>9Hz) Temporal slow delta : Occur in short sequences (bilaterally or unilaterally) Occipital slow delta : Monophasic, smooth with superimposed fast (5-9Hz) Burst of sharp theta : Diffuse or temporally	Not observed
26 – 27	Discontinuous IBI: <60s Burst : >50µV (often >300 µV) at <80s – 0.3 – 1Hz Hypersynchronous	***STOPs*** : (as above) ***Occipital Sawtooth*** : (as above) ***Theta waves*** : (as above) ***Premature temporal theta (PTT)*** : Starting to appear ***Diphasic Delta*** : >300µV at 0.3 – 1Hz  PTT	Central slow delta Occipital slow delta : High amplitude, smooth or with sparse theta/alpha superimposed Theta : Diffuse or temporally	Not observed
28 – 29	Discontinuous IBI : <30s Burst : 0.3 – 1Hz & sometimes >300µV Hypersynchronous	***PTT*** : (as above) ***Delta brushes***: start to appear : Delta with superimposed fast activity of 10 – 20Hz ***Theta waves*** : Temporal or Occipital – synchronised diffuse bursts ***Diphasic Delta*** : 30 – 300µV at 0.5 – 2Hz  Delta Brush	Central Slow Delta : abundant lasting more than 1s Delta waves less diffuse than previously – occipital predominance lasting <20s Theta : mainly temporal & occipital at 20 – 260µV (can appear sharp temporally)	AS – QS Outlined

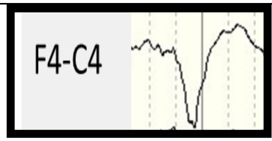
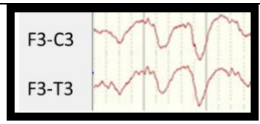
30 – 32	Discontinuous (QS) & Semicontinuous (AS) IBI: ≤20s in QS Burst: 0.5 – 1.5Hz, 100-200µV can have superimposed brushes	***PTT*** : (as above) but more in QS ***Delta Brushes*** : Diffuse 0.5 – 1.5Hz ***Theta*** : > 25µV; mainly Temporally and in QS ***Diphasic Delta*** : (as above)	Delta : 0.7-2Hz 100-200µV; mainly O-T and synchronous; more numerous in AS	Poorly differentiated AS & QS
33 – 34	Discontinuous (QS) & Continuous (AS) IBI : ≤10-15s in QS Burst : (as above)	***PTT*** : disappears in QS at 33-34w ***Delta Brushes*** : decrease in amplitude & increase in frequency (1 to 2 Hz). Occipital predominance at 34w. ***Theta*** : Diffuse ***Frontal transient*** : at 34w – often smooth, incomplete and asymmetrical  Frontal transient	Delta : Occipitally & diffuse in QS	More definite AS & QS periods
35 - 36	Discontinuous (QS) & Continuous (AS) IBI : QS <10s Burst : (as above)	***Delta brushes*** : both in AS and QS ***Theta*** : (as above) ***Slow anterior dysrhythmia (SAD)*** : short bursts monomorphic/polymorphic delta waves, 1-3 Hz, amplitude of 50-100 µV in frontal areas appears in AS 1.  SAD	Delta : 1-2Hz decreased form 100-200µV; predominant occipitally during AS; quite diffuse during QS.	QS,AS and wakefulness

TABLE 1-3 MATURATION OF THE BACKGROUND, EEG FEATURES AND BEHAVIOURAL STATES OF PRETERM INFANTS. GATHERED INFORMATION FROM ARTICLES BY ANDRE ET AL., VECCHIERINI ET AL. AND A BOOK CHAPTER BY BOYLAN.GB, LED TO THE CREATION OF THIS TABLE.

1.6.1. Influence of prematurity

EEG activity in preterm infants changes with age, and is believed to change in parallel with the anatomical development of the brain. The increased activity of the superficial cortical neurones in layer III/IV possibly represents the EEG changes mainly evident over the sensorimotor cortex (15). A prominent feature of the preterm EEG is the discontinuous pattern. It is believed that the high concentration of chloride in immature neurons cause a depolarizing postsynaptic response. This sudden change of electrical activity is thought to give rise to the burst of EEG activity (187). During the process of neuronal maturation in brain development, the chloride-regulating molecules experience expression change, which gradually makes GABA more hyperpolarizing (187). Consequently, the electrical activity progressively becomes more continuous, with the disappearance of discontinuity. It has been suggested that during the maturation of the brain from 24 weeks to 40 weeks, the bursts amplitude decreases due to gyration, which spreads the cortical electrical field (187).

The preterm brain exhibits electrical activity of very slow frequencies that can be filtered with the conventional high-pass filtered EEG, generally set at 0.5Hz (203). Preterm EEGs can be recorded with the high-pass filter set at direct current (DC) level if using these DC-coupled EEG amplifiers which highlight features of low frequency bands (0.1 – 0.5 Hz) intermixed with higher frequency bands (204). These multiband events are cortical activity clearly evident in preterm EEG when frequency filter bands are applied, as seen in figure 1-17.

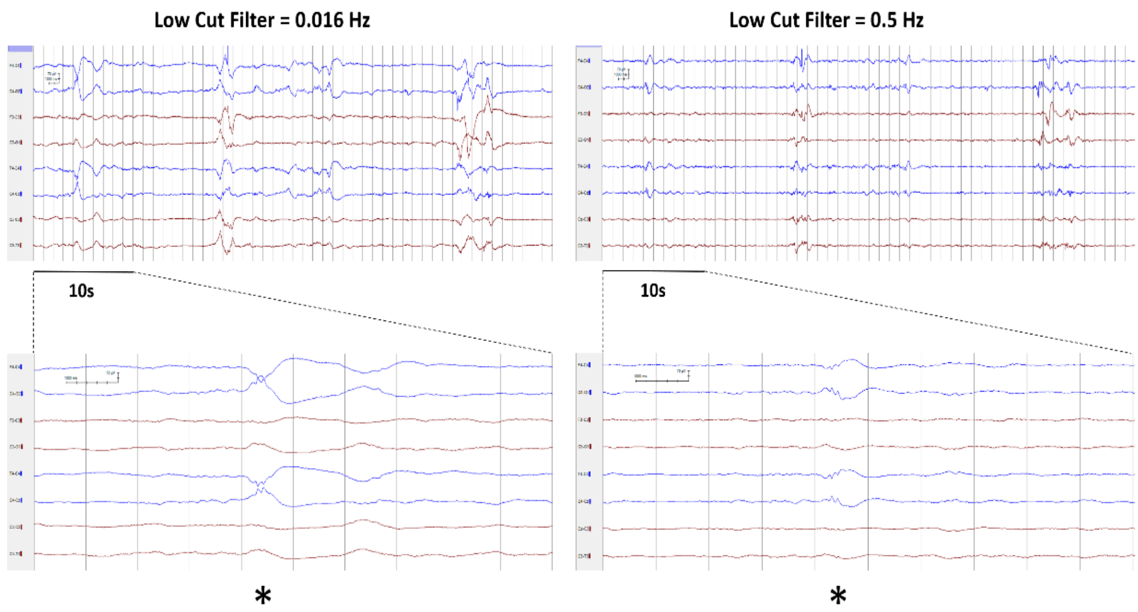


Figure 1-17 Spontaneous activity transients (SATs) recorded from DC-coupled EEG amplifiers (204).

These transients are often described as bursts or delta brushes, however filtering the lower frequencies eliminates the slower waves of the SATs, while adjusting the timebase highlights that these patterns are not genuine oscillations (187). As the brain matures, the discontinuous EEG pattern along with the SATs becomes more continuous and the SATs gradually disappears. These bursts tend to appear over the primary sensory cortices at a premature age, during the same period as when thalamocortical connections extend from the subplate into the cortical plate (187).

Animal studies have also shown that this spontaneous pattern is evident during development of the brain. A study reported that removal of the subplate neurons prevented the development of thalamo-cortical and cortio-cortical connections, which subsequently affected accurate wiring of the brain (205). Subplate neurons are found in the WM of the immature cortex and contribute to the generation of spontaneous spindle bursts. They play a pivotal role in the network of cortical activity and consequently in cortical development, by amplifying thalamic input to the cerebral cortex (205, 206). Thus, cortical activity is crucial for cortical development, and any abnormal cortical activity or disruption of subplate activity could have implications on the wiring of the brain (207, 208). Without thalamo-cortical and cortio-cortical networks, EEG signals cannot be generated (187). Cortico-cortical connections are believed to be important for the production of slow EEG waveforms, with cortico-thalamic loops and cortico-cortical connections synchronising slow oscillations (0.5Hz) from the cortex and the conventional delta waves (1 -4 Hz) from the thalamus to produce detectable slow EEG activity (209). Additionally, the maturation of intra and interhemispheric cortico-cortical connectivity coincides with the increased appearance of more mature delta waveforms (209). A recent study investigated the influence of temporal theta of the preterm EEG and reported that neural development in the perisylvian areas had an influence on preterm brain networks and the functional organization of the preterm EEG (210). An additional study has shown that large amounts of SATs activity in the first postnatal days is associated with faster growth of brain structures, proving that cortical networks are important for brain development (211). This was confirmed in a study where MRI and EEG were used to compare the rate of growth during the early postnatal period. Results showed the increased cortical network activity in the first postnatal days was important for brain growth, while total brain volume grew faster in infants with more SATs and less electrically inactive periods (212). Another study discovered that spontaneous movements of a rodent triggered sensory feedback, resulting in evoked spindle burst activities in the immature primary somatosensory cortex. It has been suggested that spindle bursts in a newborn rodent show many similarities to delta brushes of a preterm infant (213).

Synchronisation between nested oscillations within SATs and co-occurrence of SAT events is believed to initiate the relationship between populations of neurons within the preterm brain (204). As the immature brain matures, cortico-cortical and thalamo-cortical connections continue to grow within the subplate, however they are believed to grow differently in each hemisphere. This leads to asynchronous SAT activity with unilateral activities often evident. Therefore, synchrony of the brain activity relates to the early networking and can be used as a guide for brain development (214). By full-term age, the SATs activities between the hemispheres appear more synchronous, due to the formation of the corpus callosum, which allows communication between the hemispheres (187). Tracking the EEG synchrony changes during the early preterm age through to full-term age provides information regarding brain development and whether any structural or functional abnormalities are evident.

An ongoing area of research is the comparison between intra-uterine and extra-uterine maturation influence on the preterm EEG. It is unclear whether preterm infants develop EEG patterns during extra-uterine life in the same way as intra-uterine. Studies have therefore investigated the aEEG/EEG activity of preterm infants at term age and comparing to full-term born infants. Results differ between studies, with some suggesting that there is no difference (215-217), some suggesting that it delays maturation (218-220), others suggest that it accelerates maturation (221-223), while another suggested an increased incidence of premature patterns such as delta brushes (224). The most recent study on this topic compared sleep-EEG at 40-week GA between 20 preterm infants (<32GA) and full-term infants (>37GA). With the use of power spectrum and topographical analysis, it was found that by 3 months, major brain developments had occurred in both full-term and preterm infants, however the preterm infants' maturation was altered and delayed. Specifically, the immature EEG demonstrated more temporal than central activity at term age, and more occipital than central at 3 months of age (220).

1.6.2. Influence of Genetics and Environment

Early brain development such as neuronal migration and connectivity are dependent on specific epigenetic gene regulation, through DNA methylation and histone modifications, which consequently effects brain function and EEG activity (225-227). Early processes that occur between 12 – 17 weeks GA, such as neuronal migration, synaptogenesis and apoptosis are influenced by genetic factors, meaning that neuronal connectivity, in addition to genotypes, differs between infants (228). The development of thalamo-cortical and cortico-cortical connections in the subplate zone are all influenced by genetic regulation. Therefore, genetic factors influencing brain development will furthermore indirectly influence the EEG activity.

Despite the influence of genetic factors, the developing brain is heavily exposed to external stimuli. The blood brain barrier (BBB) is still developing, making the brain susceptible to toxins and insults which influence development (229). Prenatally, the most common source of external substance is from the mother, such as food, tobacco smoke or medication, which can affect the course of fetal development (230, 231). Another external source which could influence brain development in the prenatal period is maternal stress (231). Reports suggest an association between maternal stress and certain psychological disorders such as attention deficit depression, schizophrenia and hyperactivity disorder (232, 233). The transition from the womb to the external NICU environment during this critical period may influence brain development, especially as a preterm infant. One study reported how exposure to stressful procedures such as intubation, is associated with decreased brain width in the frontal and parietal lobes, altered functional connectivity in the temporal lobe and altered diffusion measures of the brain (234). Other factors in the general environment of a NICU can also influence brain development, such as painful exposures or procedures (235) noise levels (236) or NICU design such as open plan or single bays (237). This particular study recorded aEEG at 4 time-points during the neonatal stay and discovered that infants in private rooms demonstrated a trend of lower Burdjalov cerebral maturation scores at term equivalent age (237).

1.6.3. Influence of Medication

When analysing EEG recordings of preterm infants, it is essential to be aware of any medications administered. Different types of medication may influence the EEG in different ways and the EEG should be interpreted in the context of these drugs. As previously stated, surfactant is often administered to preterm infants in the NICU, due to the lack of natural surfactant in their undeveloped lungs. Surfactants cross the BBB, which means that the brain will be influenced by the drug (238). A study in which 23 preterm infants were treated with surfactant reported that aEEG depression occurred 10 minutes following treatment, with the burst rate decreasing to 67% of the initial value for a duration of 2 hours after treatment (239). The cause of this is still unclear, with no relation to transient hypotension or changes in blood gas, however there is an association with increased cerebral blood volume (240).

Caffeine citrate is used as a central respiratory stimulant of preterm infants, suffering from apnea of prematurity. Administration will also affect the EEG, however the opposite effect is evident here, where the amplitude and periods of continuity can increase. This could be explained by caffeine’s stimulatory pharmacological affect. This increased continuity can persist for up to 2 hours following the administration (241, 242). Caffeine is highly hydrophobic and can penetrate cellular membranes very easily, by simple diffusion carrier-mediated transport (243). A recent study by Vesoulis found a trend towards increase seizure burden in very preterm infants who received early high doses (30 - 80 mg/kg over 36 hours) of caffeine (244). However, this dose is larger than the range of doses typically administered clinically, which is generally between 5-10mg/kg. Contrary to these reports, a young adult study by Dworetzky et al. concluded caffeine intake is not associated with seizures, whereas smoking cigarettes increased the risk of possible seizures (245). Furthermore, a recent systematic review, also reported the seizure susceptibility relationship with caffeine, however they also reported that caffeine use in animal studies decreased the anti-epileptic effect of some AEDs, however further human studies are needed to identify acceptable dosage levels. Currently, if caffeine is administered when AEDs such as topiramate are also administered, seizure management should be closely supervised (246).

Profound suppression of the background EEG has been observed in full term and preterm infants following infusion of morphine (247, 248), whilst in a detailed preterm infant study, a burst suppression pattern became apparent following a bolus dose of morphine (249). Specifically, it was shown that cyclicity was abolished for 24 hours, while the IBI was also increased (248). Morphine has an affinity for μ-opioid receptors found in several areas of CNS including layer IV of the cortex, and enters the CNS via the BBB, before influencing brain activity (250). Another opiate drug, fentanyl, was investigated in an animal experiment looking at SATs at prematurity. This study found reduced SAT length in response to Fentanyl (251). AEDs have also been shown to have an effect on the EEG. As previously mentioned phenobarbitone, as an example is a GABA-mediated inhibitory neurotransmitter, which delays the GABA-mediated Cl^- channels from closing and consequently prevents synaptic excitability. A study by Shany et al. examined the influence of various AEDs on the aEEG (inc. lorazepam, diazepam, phenobarbitone, midazolam and lidocaine), assessing the voltage changes before and after administration, in addition to the time taken to return to initial voltage (252). Significant depression was evident following administration of lorazepam, diazepam, phenobarbitone and midazolam. The recovery time in these AEDs ranged from 15min – 15hrs, with an average period of 2.5 hours. Thus, drug dosages affect the EEG recovery time, but this is also dependent on the AED, as different AEDs have different half-lives. The AED with the longest half-life is phenobarbitone, which lasts for 48 – 147 hours, depending on the dosage (253).

1.7. Amplitude-integrated EEG (aEEG)

The aEEG is derived from the EEG. It is a bedside tool, used mainly in ICU, which records from 1-2 EEG channels. These signals are filtered, rectified, processed, and displayed on an amplitude and time-compressed scale, to represent a time-compressed measurement of EEG. This allows changes in EEG amplitude and activity over long durations to be viewed on one page of a screen, however information concerning the EEG waveforms is lost. Maynard et al. developed this form of cerebral function monitor in the 1960s as a way to monitor neurological function over time in comatosed adult patients (254). The raw EEG signal is recorded from the electrodes, and initially amplified before passing through asymmetric pass-band filters that attenuate activity less than 2Hz and above 15Hz (240). The reason for this is to remove artefacts from the recording. The signal is rectified and smoothed before undergoing time compression and semi-logarithmic amplitude compression (255). This allows the aEEG to be generated, by displaying amplitude fluctuations over a short period of time. Modern aEEG devices have the advantage of displaying both the aEEG trace and the raw EEG trace. It has been adopted by neonatologists and it has the advantage of being a simple and user-friendly method to monitor brain activity in neonates. It has some value in detecting seizures, as seizures can cause transient deflections on the aEEG, but it must be checked against the raw EEG trace as other factors such as transient artefact can produce similar transient aEEG deflections. As aEEG is only recorded from a small number of electrodes, usually in the fronto central or parietal regions, focal seizures in other areas may be missed compared to EEG monitoring with a fuller electrode coverage of the head (256). EEG remains the gold standard for seizure detection.

To record a single channel aEEG, the international 10-20 classification suggests using a pair of biparietal electrodes over the P3-P4 positions (257). Nevertheless, other positions such as C3-C4 are acceptable. Commonly, two channel aEEG is performed, where F4-C4 and F3-C3 are the preferred positions in the NICU at CUMH.

1.7.1. Preterm aEEG

The aEEG of the preterm infant is predominantly discontinuous, demonstrating a burst – interburst interval (IBI) pattern called Trace Discontinu. This is a normal physiological state and should not be confused with the pathological burst suppression pattern. IBIs are quiescent EEG periods reflecting low voltage brain activity (258). The lower margin of the aEEG is defined by the lower amplitude range of the EEG while the upper border is defined by the peak amplitude of the bursts (259). By 34-week GA, the EEG should be continuous, and this should be reflected in the aEEG recording. Figure 1 – 18 shows how an aEEG of a preterm infant initially displays a discontinuous aEEG recording, and becoming more continuous by 34 weeks GA. Also evident in this figure is sleep cycling or cyclicity.

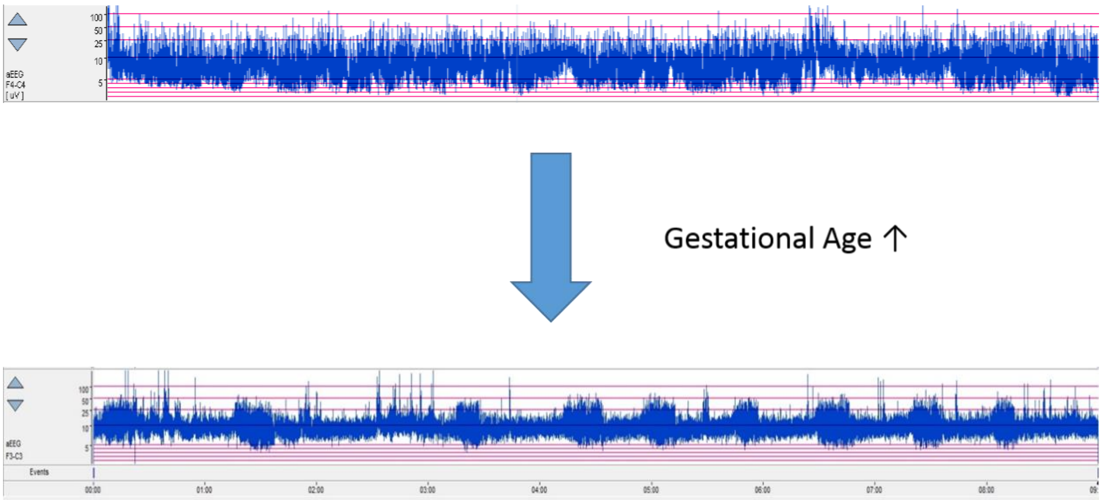


Figure 1-18 EEG discontinuity change with age. Illustrates how the aEEG is initially discontinuous in a preterm infant (27 weeks GA), but becomes continuous by 34 weeks GA.

The pattern aEEG classification by Hellstrom-Westas and Rosen (259) recommends that a discontinuous aEEG has a low band activity of <5 µV and upper band of activity of >10 µV, while a continuous aEEG has a low band activity of >5 µV and upper band activity of <50 µV. The full classification was described as:-

A. Continuous - Low band activity of >5 µV and upper band activity of <50 µV
B. Discontinuous – Low band activity of <5 µV and upper band of activity of >10 µV
C. Burst Suppression – Discontinuous activity with periods of very low activity <2 µV and upper band of: > 25 µV
D. Continuous Low Voltage – Very low voltage with lower band of <5 µV and upper band of <10 µV
E. Flat Trace – Extremely low voltage of <5 µV

An example of the different aEEG classifications are shown below:-

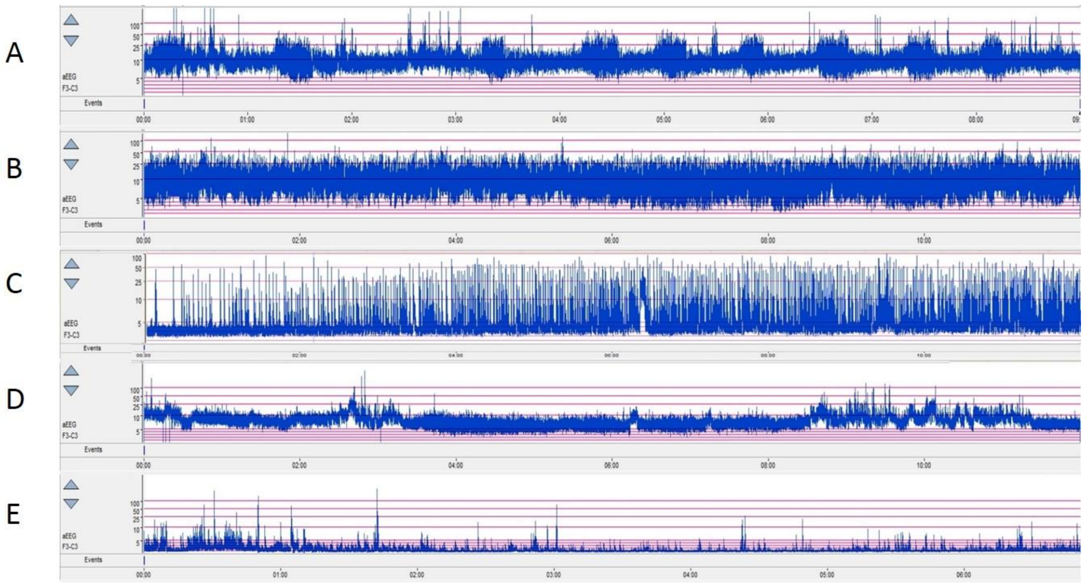


Figure 1-19 aEEG classification by Hellestrom-Westas and Rosen. A, Continuous (nearer term age of 34 weeks GA); B, Discontinuous; C, Burst Suppression; D, Continuous Low Voltage; E, Flat Trace.

A method of scoring the background activity of the aEEG was suggested by Burdjalov et al., by distinguishing certain features of the aEEG (Figure 1-20) (260). This method was designed for full term and preterm infants. Four components are estimated from inspecting the aEEG, namely the continuity, the cyclicity, the lower border amplitude, and bandwidth span and lower border amplitude (260).

1. Continuity is assessed by the overall density of the trace, with frequency variations over time. This can categorise the aEEG as continuous or discontinuous normal voltage.
2. Cyclicity is when the bandwidth expands and contracts due to the state of the infant. Sleep-wake cycling is evident when the aEEG displays waxing and waning morphology.
3. Amplitude of lower border is the average lower amplitude level during the recording epoch.
4. Bandwidth is the difference between the upper and lower borders.

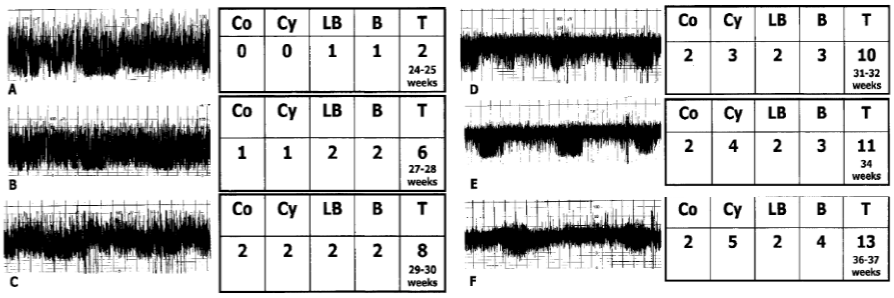


Figure 1-20 Burdjalov aEEG background scoring system; reprinted from Burdjalov, VF et al. Cerebral function monitoring: a new scoring system for the evaluation of brain maturation in neonates (260).

There are differences between the two classifications, however the main one is that the Burdjalov score only indirectly provides measures for pathological patterns with the primary focus of describing the physiological maturation of electrocortical activity. In comparison, the Hellström-Westas classification distinguishes pathological and physiological pattern. A study by Burns et al. investigated the two classifications and their performance in predicting prognosis, and although both appeared useful, the Hellstrom Westas classification performed better for the prediction of outcome at 2 years of age (261). Although the Burdjalov scoring system is commonly used, it does have its limitations. First and foremost, it only looks at the aEEG alone and does not refer to the raw EEG at all. Rule number one for aEEG interpretation is that the raw cEEG should also be investigated, even if it is only one or two channels. It is very important to look at the raw EEG concurrently with the aEEG, even if limited channels are available, as some artefacts can only truly be identified by the raw EEG, such as movement or muscle artefact. Furthermore, the summarization of the descriptive scores are vague and subjective. An example of this are the descriptions ‘somewhat continuous’ and ‘somewhat cyclical’. The word ‘somewhat’ is a vague which could lead to inconsistent results between reviewers. Another limitation is the similarity between the scores of amplitude of the lower border category. There are three possible scores and only 2µV separates the lowest score of 0 and having the highest score possible of 2. Having such a small difference to separate three possible scores questions the relevance of the lower border amplitude category. This scoring system is, however, a readily available scoring system that has been used in numerous aEEG investigations in preterm infants (262-268).

The background scores of preterm infants depend on the GA, with discontinuity being more pronounced in the more extremely preterm infants while cyclicity becomes more apparent in the older infants. The aEEG is often used for preterm infants because of ease of application, maintenance, and interpretation (259). It is a user-friendly procedure that is used worldwide in the NICUs. Nevertheless, research has shown that it is not as accurate as the EEG, providing lower seizure detection sensitivity and inter-observer agreement (269, 270). If the aEEG is used as a diagnostic tool, it is strongly advised that the raw EEG should also be interpreted to confirm any findings (271).

1.8. Abnormal Preterm EEG– Short term diagnosis

The presence of normal or abnormal waveforms on the EEG can also be used to assess brain development in premature infants, as well as being the gold standard for the detection of seizures.

Certain EEG patterns have been related to CRUS structural abnormalities, such as positive rolandic sharp waves (PRS) and white matter injury (272-274). Additionally, it is possible for the spatial and temporal organization of EEG patterns to be disrupted, which may reflect impaired brain development (194). As a result of these findings, Watanabe et al. characterised abnormal EEG features into acute and chronic stage abnormalities (ASA and CSA, discussed below) (194). Further studies used the Watanabe classification to study brain injury in preterm infants, such as the association with PVL, with one study showing that 96% of infants suffering from PVL presented abnormal EEG traces (275). It is thought that the timing of brain injury can be estimated due to the ASA/CSA features of the EEG (Figure 1-21) (194). The EEG pattern of an acute brain injury is an initial ASA (or EEG depression), followed by a gradual improvement of the ASA, before being replaced by CSA waveforms. Watanabe suggests that the first EEG after birth can provide information about whether an injury was prenatal or perinatal. In a situation of prenatal injury, EEG monitoring would miss the initial ASA, therefore only displaying CSA. A perinatal injury would therefore show ASA on the EEG if monitoring occurred soon after birth, while a postnatal injury would display ASA later (194).

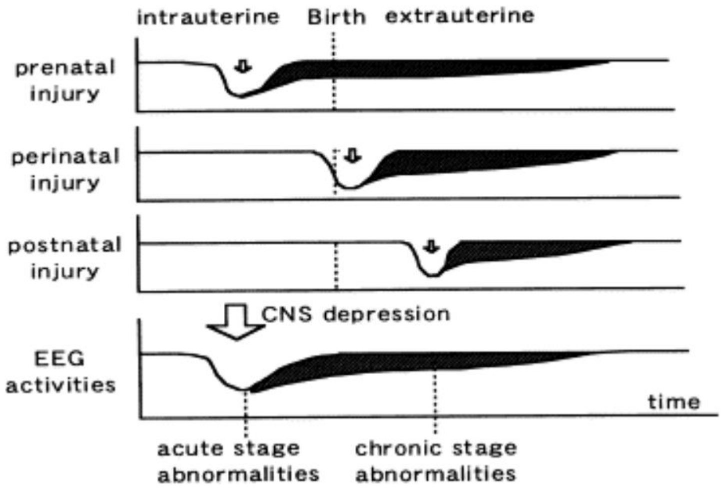


Figure 1-21 The timing of brain insults and impact on EEG findings ; reprinted from Watanabe et al. (194)

<u>Acute stage Abnormalities</u>

Acute stage abnormalities indicate a change in cerebral function or more specifically characterised by suppression of normal background activity (194). Common changes are the disruption of normal sleep- wake cycling, decreased continuity for the expected GA, or evidence of voltage decrease. These changes have been reported to occur as a result of primary brain injury, such as in a response to an acute IVH (276). Alternatively, these disruptions could temporarily occur secondary to factors such as surfactant, sedative or antiepileptic medication (239, 249, 251, 277), or alternatively secondary to clinical events such as low cerebral blood flow (278). The severity of the ASA is graded by five scores, with 5 being the most severe (Table 1-4).

	Continuity	Frequency	Voltage
ASA Grade I	Prolonged IBI	Attenuated α, β, θ	
ASA Grade II			Mildly low voltage
ASA Grade III	Decreased continuity		
ASA Grade IV	Absent continuity	Only Delta activity	Moderately low voltage
ASA Grade V			Very low/flat voltage

TABLE 1-4 WATANABE CLASSIFICATION OF ACUTE STAGE ABNORMALITIES.

Chronic stage Abnormalities

CSA are classified as either disorganized or dysmature EEG patterns, and are graded as mild, moderate or severe. Some features of both patterns may also be present simultaneously over a number of time-periods (194) and prolonged monitoring or serial EEGs are recommended to assess the evolution of the EEG and characterize the pattern type.

Disorganised pattern

Disorganised waveforms are abnormal and morphologically deformed, presenting with lack of 'smoothness', wider base, increased peak-to-peak amplitude, abnormal sharp waves and delta brush activity with spiky, cogwheel-shaped appearance. These are often evident in delta waveforms and/or delta brushes known as 'mechanical brushes' (194), which have specifically been associated to PVL, reflecting also the side of the lesion (279). In terms of short term diagnosis, disorganised waveforms have reportedly been associated with strong/acute injuries to the brain, such as severe perinatal asphyxia or severe IVH (194, 280, 281). A study by Okumura has shown that CSA was useful for assessing brain injury, with disorganised abnormalities evident in 60% (31 of 52) of infants with PVL and 13% with IVH. (280). The severity of disorganised waveforms depends on whether they appear occasionally or whether they are evident during the whole tracing.

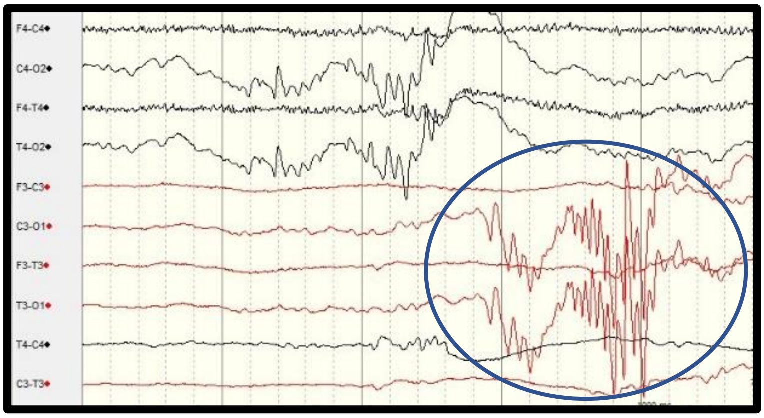


Figure 1-22 Example of Disorganised patterns. Infant 31+6 GA, with an EEG recording started at 4 h of age. CRUS demonstrated asymmetry of the lateral ventricles (left > right), while the MRI showed resolving left grade 1 IVH.

Dysmature pattern

A dysmature EEG is when immature patterns, or IBI durations typical of a younger GA are evident in a more mature preterm infant. These include EEG patterns and transients such as very high amplitude delta activity, temporal theta bursts or rhythmical activities that suggest a younger EEG pattern than the infant's actual corrected age (194). For this activity to be properly categorised as a CSA and not acute depression, grading should be made closer to term age, where its persistency should be evident, therefore prolonged or serial EEGs are recommended for an accurate interpretation (224). In terms of short-term diagnosis, dysmature patterns are reportedly associated with mild/prolonged brain injuries (194, 280, 282). Associations could be from conditions causing mild, prolonged depression of central nervous system function, such as patent ductus arteriosus and CLD including BPD, or ROP (283, 284). In the study mentioned previously in the disorganised pattern section by Okumura, it was also reported that dysmature patterns were also seen in 28 infants, with 11 providing evidence of IVH and 1 with PVL (280).
Furthermore, there has been some ambiguity around this classification system, as dysmature features are also evident in the ASA and are also intermingled with more disorganized patterns (283). A feature regarded as a dysmature pattern by some authors is asynchrony, however this is an accepted feature in very and extremely preterm infants. Its presence in infants ≥ 28 week GA, might be a sign of abnormal

maturation (174, 176), such as alteration in subcortical signalling and of progressive synaptogenesis in the corpus callosum (174, 190).

Figure 1-23 Example of immature Dysmature patterns Infant 31+2 GA with an EEG recording at 32 weeks' GA. This shows mild features of immaturity, namely delta waves and brushes which are normal in morphology but still diffuse and anterior, instead of localized in occipito-temporal regions.

Positive Rolandic Sharp Waves (PRS)

PRSs (figure 1-24) are sharp activity of positive polarity appearing in the rolandic/central region of the brain. This transient has been classified into two types: type A and B (285). PRS type A have a fairly high amplitude, a clearer association with WMI and are clearly evident on the background EEG, while type B are of lower amplitude, consequently harder to identify and the prognostic significance is less understood. It is a pattern first described by Dreyfus Brisac and Cukier in 1972 (286), and was originally reported in association with IVH (287-290). This association developed, where PRS was believed to be a specific marker for WMI or PVL (291, 292). Reported incidence rate of 43 – 100%, along with an association with disorganised EEG patterns, suggest that these waveforms could provide early markers for PVL detection. As the incidence of PVL has decreased, so too has the frequency with which we encounter PRSs in the preterm EEG (293). However, uncertainty revolves around this area with one study suggesting that PRS rarely detects PVL, and that ASA and CSA severity correlates better with severity of PVL between days 1 – 4 and 5 – 14 of age, respectively (294).

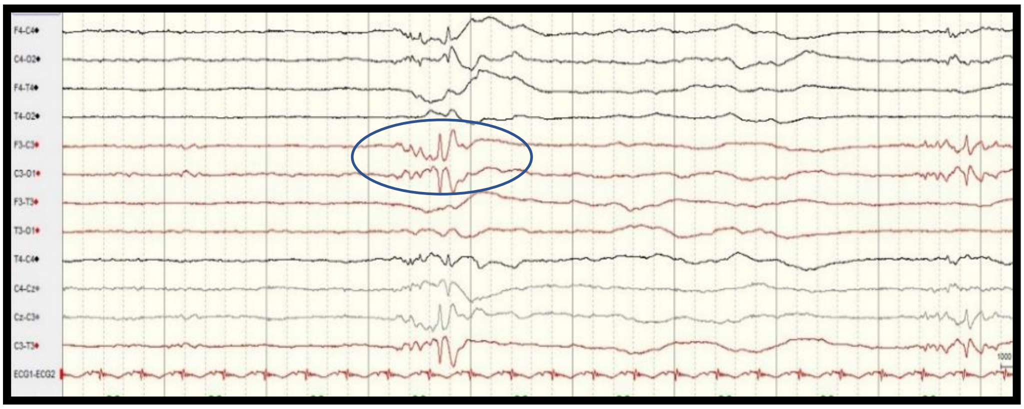


Figure 1-24 Example of Positive Rolandic Sharp Waves. Infant 26+2 GA with an EEG recording that started on the 2nd day of age. CRUS showed grade 1 IVH.

<u>**Positive Temporal Sharp Waves (PTS)**</u>

These waves are very similar to PRS, but localised over the temporal regions. Their prognostic significance is unclear, however one study has reported that a frequent occurrence could be associated with poor outcome (295). In contrast, it has been reported that when the occurrence is less in number, short in duration, low in amplitude and regress rapidly, the association with poor outcome is not significant. Additionally, PTSs (figure 1-25) appeared less and decreased rapidly in healthy infants, while appeared more and persisted for longer in infants with pathological complications (296). Identification of this transient can sometimes be difficult, as it is similar to the normal PTT transient (277, 297).

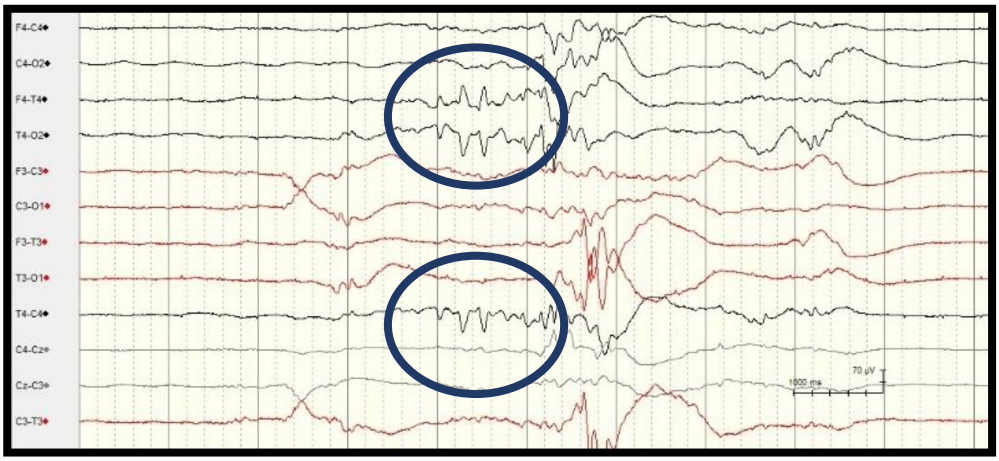


Figure 1-25 Example of Positive Temporal Sharp Waves. Infant 31+6 GA with an EEG recording at 4 hours and 18 minutes of age. CRUS was normal.

Mechanical/abnormal brushes

These waveforms are defined as spindle-like fast wave activity of frequencies between 13 and 20 Hz and with maximal amplitudes over 40 µV (figure 1-26). The spindles appear more pronounced and sharper compared to normal delta brushes. A simple approach of recognising these waveforms is by applying a low cut filter of 10 Hz in order to eliminate slow waves (279). They are most often visible over the occipital-temporal and central regions in infants with PVL, reflecting the side of the lesion (279).

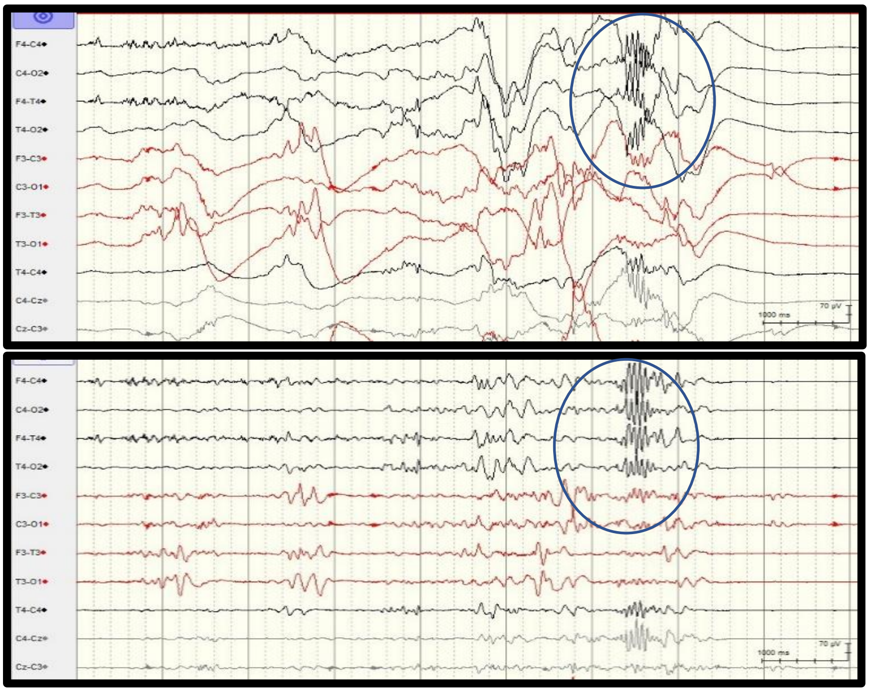


Figure 1-26 Example of a mechanical/abnormal brushes before and after filtering. ***Infant 24+6 GA with an EEG recording at 4 hours and 51 minutes of age. CRUS showed right IVH-IV. The parenchymal changes involve the posterior frontal lobe and measure approximately 1 cm from anterior to posterior. This infant did not survive.***

Asymmetry

The background activity of the EEG is expected to be symmetrical across both cerebral hemispheres, in terms of amplitude, frequency and morphology. When the EEG is asymmetrical (figure 1-27), it may reflect an underlying brain injury. There is usually a certain degree of asymmetry that is considered normal (if less than 50%

between the 2 hemispheres), and a right side predominance of temporal delta waves has been reported as normal at certain ages (175).

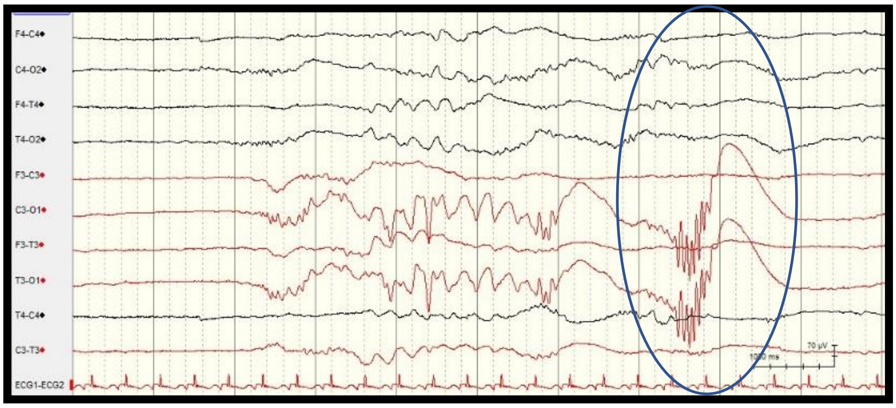


Figure 1-27 Example of an asymmetry. Infant 31+6 GA, with an EEG recording started at 4-h of age. CRUS demonstrated asymmetry of the lateral ventricles (left > right), while the MRI showed resolving left grade 1 IVH.)

Asynchrony

Synchrony of the EEG is evident when specific waveforms and transients occur across both hemispheres without a time delay. Asynchrony is considered a normal feature of very and extremely preterm infants, however if high amplitude bursts appear consistently asynchronous in infants ≥ 28 GA, during bursts of temporo-occipital delta activity in those between 30-34 GA and if present during AS in infants aged 35-36 weeks, it can be a sign of abnormal maturation. (175, 176, 298). As the corpus callosum facilitates communication between the hemispheres, it is believed that reduction in asynchrony of the EEG with GA, reflects progressive synaptogenesis of the corpus callosum, in addition to the theory that asynchrony reflects alteration in thalamic subcortical signalling (190, 298).

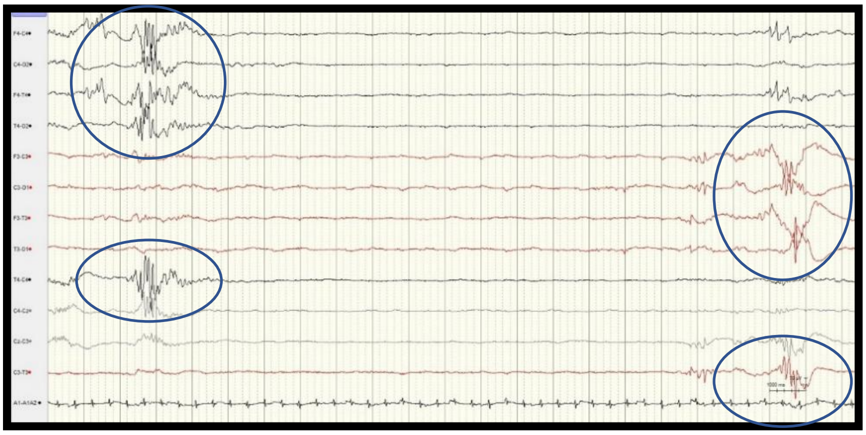


Figure 1-28 Example of asynchrony Infant 34+6 GA with an EEG recording at 4 hours of age. MRI showed focal ischaemic change in the right ventrolateral thalamus/posterior putamen.

1.9. Abnormal Preterm aEEG– Short term diagnosis

The aEEG is commonly used in the NICU, and research to discover if any patterns are related to short term outcome has been undertaken. The classification by Hellstrom-Westas and Rosen, in addition to the scoring system by Burdjalov et al. have be used to study the aEEG of preterm infants with significant brain injuries. The most prominent feature of the aEEG to identify significant brain injury is the absence of sleep cyclicity (255, 299-301), as evident in figure 1 – 29.

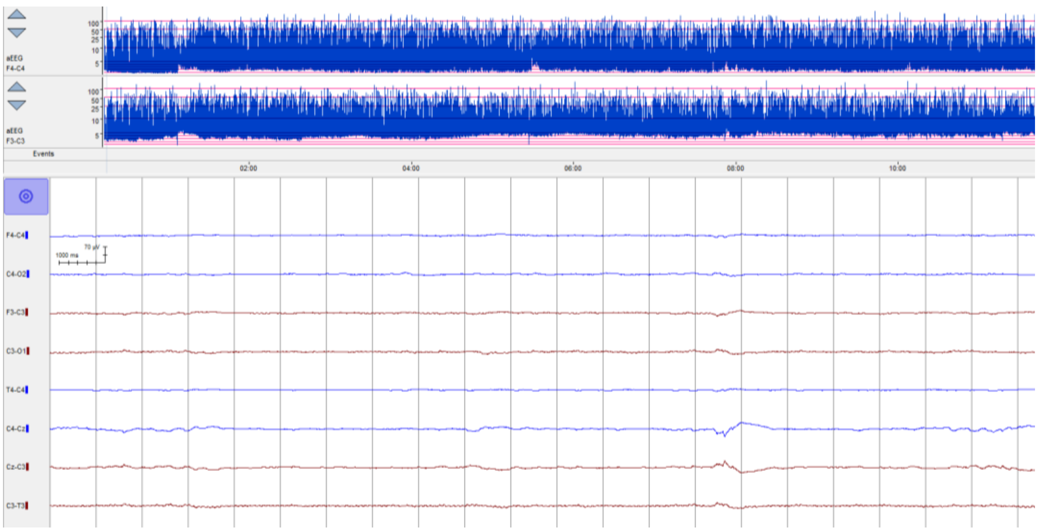


Figure 1-29 Absence of aEEG sleep wake cycling in a 30+0 male infant with grade IV intraventricular haemorrhage.

In a study by Olischar et al., two additional features were prominent in infants with IVH, which were increased seizures captured from the aEEG and increased discontinuity (Figure 1-30) (299).

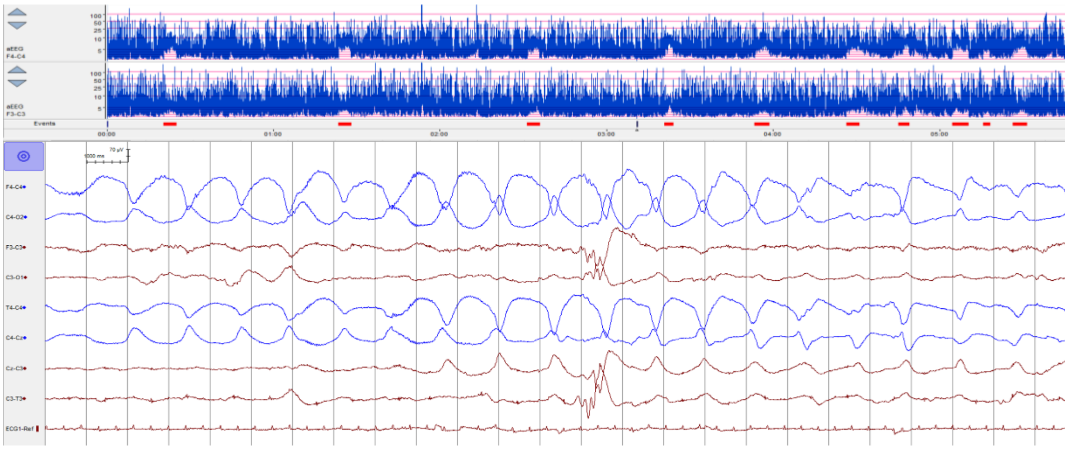


Figure 1-30 Increased discontinuity and seizures seen on aEEG and EEG in an 30+4 GA male infant with right sided grade IV and left sided grade II intraventricular haemorrhage.

Both the classification by Hellstrom Westas and Rosen and Burdjalov scoring system was used by Soubasi et al. for the identification of infants with IVH, with numerous aEEG features showing associations with the injury: burst suppression, flat aEEG trace and voltage decrease of discontinuity and continuous patterns (302). Voltage decrease was also reported by Wikstrom et al. in infants with IVH and WMI (303). In reality, although the research is interesting, clinically aEEG is not being used for the identification of severe IVH due to the other available neuroimaging tools.

1.10. Preterm EEG/aEEG as a prognostic tool

Preterm infants are at increased risk of neurodevelopmental abnormalities. The aetiology is multifactorial, but brain injury in the form of IVH and PVL are two of the leading causes. Many other factors can impact brain growth and development at this crucial time in maturation including malnutrition, sepsis, NEC and BDP. It is therefore imperative to improve our knowledge regarding the preterm brain and to do so, research needs to continue in the area. Significant advances over the last three decades, such as nutrition, respiratory support and maintenance of temperature have seen a reduction in the incidence of many of these conditions, with a relative improvement in survival and developmental outcome (304). Nonetheless, IVH and PVL still remain significant problems, especially in the more immature infants who remain at greatest risk of adverse short and long-term problems. Therefore, studies investigating the preterm brain and the preterm EEG should continue to provide further useful information for long term development and to improve our current knowledge.

1.10.1. EEG Monitoring

Although EEG is the gold standard for assessing brain function, the procedure or application per study can differ, such as minimising the amount of electrodes used, or the performance of serial EEG recordings. Appendix E indicates the identified articles that used EEG as a long-term prognostic tool.

EEG performed with limited electrodes is common, and the disadvantage of this is limited spatial data. Availability of clinical staff to interpret conventional EEG can often be difficult, therefore it is not surprising that there is an over reliance on the aEEG trend in the NICU. A study by West et al used EEG continuity measures from two channel EEG, within the first 48 hours after birth, to predict outcome in a cohort of infants below 29 weeks (305). Results showed that although quantitative analysis of EEG continuity had the potential to correctly predict neurodevelopmental outcome, it was not as accurate as a neurophysiologist's assessment. The evaluation of IBI, burst features, seizures and synchrony was assessed by the neurophysiologist, while the quantitative analysis of continuity was determined by the percentage of the amplitude of the EEG reaching a specified amplitude threshold in one minute. A limitation was that only 1 hour of EEG was analysed. Although limited periods were used for both assessments, using more EEG data might have improved the EEG evaluation. Some studies investigate particular features of the EEG, such as bursts, and this can sometimes be achieved from limited channels. A study by Iyer et al., recorded 2 channel EEG of preterm infants between 22-28 weeks GA at 12, 24, 48 and 72-hour post-natal ages, to investigate the bursting pattern of cortical activity and showed that bursting properties can correlate with long-term neurodevelopmental outcome. Results also showed that higher burst sharpness and low regression slope values (linear relationship between burst area and duration) were correlated with death or poor cognitive Bayley scale scores (<85 score) at 2 years (306). A limitation of the study, which is often seen in preterm studies, is the limited number of electrodes resulting in a poor spatial coverage. This could result in missing important activity or even seizures.

Some studies have used multichannel EEG for preterm monitoring. A grading system often used to predict adverse neurodevelopmental outcome or mortality is the Watanabe grading system (194). Using this system, research has suggested that dysmature patterns of the EEG are the most prominent features for assessing the prognosis of neuromotor (307) and cognitive development (280, 307). In a study by Le Bihannic et al., it was reported that disorganised EEG led to cognitive abnormality in all cases, with two diagnosed with CP. The sensitivity and specificity of EEG within the first week of life for psychomotor outcome were 83.3% and 88%, respectively (307). High specificity in particular suggest that absence of CSA patterns is a good predictor of cognitive outcome. This is supported by another study, Hayashi-Kurahashi et al. who found a high specificity for moderate to severe abnormalities, while the study also found that disorganised patterns without PRS within the first month of life was a marker of adverse outcome (293). These findings suggest that dysmature and disorganised patterns have the potential to be an indicator for poor outcome. The acute stage abnormality (ASA) feature from the Watanabe grading system were considered in another study by Maruyama et al. who investigated the prognostic value of EEG in the development of CP. An association was described between the presence of severe ASA on day 1 or 2 and CP following a psychomotor developmental assessment at 18 months (308). This suggests that both CSA and ASA should be considered as early indicators of complications. The most significant limitation of these multichannel studies is the short length of monitoring undertaken. Majority of studies report monitoring periods of approximately 40 min – 1 hour, which is short when compared to the majority of aEEG studies that monitor for 3 days or more. In short recordings, seizures might be missed, while state change is also difficult to identify in such a short period. The early postnatal period is when an infant is vulnerable, therefore recording the whole period is imperative and monitoring a portion of this period is unreliable.

A grading system was devised by Perivier et al. to assess the association between short serial EEG recordings (minimum of 45 minutes) and neurodevelopmental outcome. Here, the devised grading system that ranked the EEG as normal, moderately abnormal, or severely abnormal was used on the basis of the degree of abnormalities and their persistence. The EEG during the first week of birth showed good specificity and positive likelihood ratio for the prediction of outcome at 2 years of age (309). In contrast, a significant limitation of the study was that although the specificity was good, the sensitivity was very poor at 16%, which raises concerns regarding the quality of the devised grading system. Furthermore, another limitation was that, although a lot of EEGs were performed, infants did not receive equal number of recordings. Certain infants might have received multiple recordings while others only received one. This means that data collected per infant is not comparable and the time and age of the infants during EEG recordings might not be comparable either. Finally, it was also reported that over 120 paediatricians performed follow-up at 2 years of age, therefore a large amount of subjectivity was also introduced to the study.

Quantitative analysis of the EEG is also thought to be useful, with total absolute band power (tABP) being used for outcome prediction. A study by Schumacher et al. found that the tABP in infants between 24-28 weeks GA was significantly lower in infants who had a poor outcome, as assessed by the Bayleys scales. Specificity and negative predictive value were high in this group, whilst no correlation was discovered between tABP and outcome in infants between 28-31 weeks GA (310). Furthermore, a study by Jennekens et al. found that most tABP bands (especially δ and θ) display maturational changes, decreasing with PMA, while relative α and β power increase. It is thought that maturational change is largest in the frontal and temporal regions, whilst the EEG change during maturity could be due to ongoing brain growth and development (311). Although quantitative analysis is improving in EEG, certain limitations were evident in these two studies. Neither studies analysed the EEGs visually, therefore complete confidence was put into the trend analysis, while certain EEG features of particular morphology would not have been identified. Schumacher et al., for example only analysed the tABP, via a review software package, meaning that no electroencephalographers were used for analysis. Furthermore, these trend softwares were designed to analyse adult EEGs, not preterm EEGs. Finally, a limitation of the Jennekens study was the very low sample size of 18 infants, however three aspects of the transformed signal was calculated, which allowed detailed analysis to be performed.

Some reviews have attempted to evaluate the usefulness of EEG monitoring in the preterm infant. An EEG based review by Hellstrom Westas and Rosen, reported a correlation between preterm EEG findings and short or long term outcome (312). It stated that the only EEG feature associated with a specific brain injury was PRS, which indicated prognosis of CP (312). Furthermore, a review by Tich et al. suggested that disorganised patterns also show a risk of CP, whilst PRS was a marker for PVL. Therefore, there are still discrepancies in the current literature. Variability is clear among studies which emphasises the need to improve the approach for studies in the future. The main variations that occurs across preterm EEG studies are the age at which the EEG is performed, the length of the EEG recordings and number of recording per patient, and finally the age of neurodevelopmental follow-up investigations. In terms of EEG, the ideal approach would be to perform the monitoring as early and for as long as possible, whereas in terms of follow-up, a study be Breeman et al. suggested that adult IQ could be predicted by cognitive assessment at 2 years of age (313). Therefore, follow-up at 2 years is a reasonable period to assess development.

1.10.2. aEEG Monitoring

Appendix E further indicates articles that used early aEEG during the first days or weeks as a long-term prognostic tool. Studies using aEEG techniques have found different results when investigating the performance of predicting neurodevelopmental outcome. Even though the Burdjalov score has numerous limitations, as previously described, it is still commonly used in aEEG studies predicting outcome (262-268). Numerous variables and heterogeneity are identified between aEEG studies, making study evaluations difficult. Length of recordings range between 4 – 72 hours, while follow-up ages differed also, ranging between 4 – 24 months (261-266, 268). Furthermore, a mixture of findings have been reported from different aEEG studies. Vesoulis et al. found that seizures in the first three days of age had an association with poor language development and death (314), while Wikstrom et al. discovered that burst suppression pattern and IBI percentage >55% at 24 hours and a depressed aEEG in first 12 hours was associated with poor outcome (315). Certain findings were reproducible however, with a second study from Wikstrom et al., reporting that aEEG depression was also associated with an adverse 2 year outcome (303). Furthermore, a study by Reynolds et al. found that the lack of cyclicity in the first 6 weeks of life, was associated with poor outcome (265), and this can be compared to a study by Kidikoro et al, that concluded that the absence of aEEG cycling was predictive of poor outcome, however this association in particular was during the first 24 hours of age in infants between 27 and 32 weeks GA (316).

Although EEG monitoring is the gold standard, it is not always possible to perform due to the difficulty of applying numerous electrodes, which consequently takes a longer time to setup. Therefore, it is also important to continue the research of aEEG and prognosis, as currently this is utilised more often than EEG in a clinical setting.

Summary of EEG/aEEG as a prognostic tool

A recent Cochrane review from Kong et al, investigated the relationship between aEEG/EEG and outcome, suggesting that both aEEG and EEG are useful tools (317). aEEG findings such as absent cyclicity, burst suppression, prolonged IBI, a generally depressed aEEG and seizures have an association with adverse neurodevelopmental outcome. In addition, EEG findings such as ASA, CSA and abnormal transients also suggested a poor prognosis.

As knowledge regarding preterm EEG increases, the need for a standardised EEG system for assessing brain health and the prediction of outcome becomes increasingly important. An EEG grading system, similar to that of the EEG in hypoxic-ischemic encephalopathy, could be very beneficial for outcome prediction.

1.11. Preterm aEEG/EEG Seizures

Seizures in infants can indicate underlying neurological dysfunction (14). Preterm infants are at risk of seizures in conjunction with brain injury during the vulnerable early postnatal period (315, 318). It is important to protect the developing brain, and treat seizures, however it is also important to avoid treating infants unnecessarily, due to the neurotoxic effects of AEDs (124, 125).

Early studies researched the occurrence of seizures based on clinical features alone, without the aid of EEG monitoring. One study collected prospective questionnaires for all infants in the NICU, following educational sessions regarding neonatal seizures and discovered an incidence rate of 11.1 per 1,000 live preterm births, with preterm infants 6 times more likely to have seizures compared to full-term infants (319). A study that retrospectively obtained information from medical records discovered a rate of 57.5 per 1,000 live births of infants <1,500 grams, which saw the rate decreased as the weight and age increased, similarly to the previous study (320). The vast repertoire of general movements of a preterm infant makes seizure diagnosis a great challenge, as true clinical seizures are often subtle and very difficult to distinguish. Therefore, seizure classification without EEG monitoring is inaccurate, due to the lack of neurophysiological evidence leading to misdiagnosis.

Seizure duration has been reported to be shorter in preterm infants compared to full-term infants. This, along with the time compressed semi-logarithmic scale of the aEEG, makes preterm seizure identification with aEEG alone very difficult (321). It is clear that multichannel EEG should be used in conjunction with aEEG to increase the accuracy of seizure identification (322).

1.11.1. EEG Monitoring

Whilst monitoring the neurological state of preterm infants in the NICU remains challenging, the potential benefits may be significant and EEG monitoring is now much more feasible (323). Appendix F summarises the studies using conventional EEG to identify preterm seizures. Although Okumura et al. studied seizures in a cohort of infants which included preterm infants up to 37 weeks GA, it was possible to identify seizure findings only from infants that were 32 weeks GA or less. From the 1045 infants recruited, 408 were 32 weeks GA or less, 9 infants had seizures and 4 were below 32 weeks (0.9%). Most seizures started in the first 4 days of age, whilst all had a focal onset, which was maximally temporal (324). Even though the main objective of a study from Le Bihannic et al. was to evaluate the prognostic value of the EEG in preterm infants, seizure frequency was also described. From 61 infants below 30 weeks GA, two infants (3%) developed seizures during the first week, from a mean recording duration of one hour (307). Interestingly, no clinical manifestations were evident during the episodes. Pisani et al. found an incidence of 8.7%, following 1-hour monitoring of infants when risk factors or clinical signs of seizures became evident (325). It was also reported that mortality in very preterm infants with seizures is double that among similar infants without seizures.

Other studies have studied seizures in preterm infants, but not specifically <32 weeks GA. An incidence of 6.1% was reported in a cohort of infants <36 weeks GA, however only one hour of EEG recording was recorded (321). Seizure onset was also reported within 48 hours in 27.4% of infants, whilst the majority of infants were <29 weeks (321). A study by Davis et al. with the same age group population found that 6.4% of infants had clinical seizures, however only 22% of these infants with clinical seizures, had confirmed EEG seizures (326). In a study by Glass et al., with a population of infants <34 weeks GA, 3.8% of infants had clinical seizures, however only one infant displayed electrographic seizures. However, only 2-hour recordings were undertaken (327).

The main limitation in current EEG studies is the lack of continuous, long term monitoring data. Although seizure frequencies are generally found to be low, studies are unconvincing as EEG recordings are sparse and of short duration.

1.11.2. aEEG Monitoring

Appendix F further displays studies that used aEEG to determine the frequency of seizures in infants below 32 weeks GA. From each study, seizure frequency was reported, where possible, which highlights the varied findings. West et al. described 5 infants (6.6%) presenting with seizures from a cohort of 76 infants below 29 weeks GA. All infants with seizures had poor outcomes, including 4 who died (305). A study by Shah et al. reported a frequency rate of 22% from a group of 51 infants below 30 weeks GA, whilst also stating that seizures were more likely to occur in sicker and more premature infants. These infants had aEEG during the first week of life for a median duration of 74 hours and poor outcome was associated with the seizure incidence (318). A seizure frequency of 43% was documented by Wikstrom et al. from a cohort of 49 infants between 22 – 30 weeks GA. As the recording commenced during the first 3 days of life, seizures had a strong association with IVH, however no association was evident with subsequent neurodevelopmental outcome (315). During a two year period, 95 infants between 24 – 30 weeks GA were included in a study by Vesoulis et al. who found seizures in 48% (314). By recording continuously as soon as possible after birth, for an average duration of 66 hours, Vesoulis stated that seizures identified on aEEG were common in the first 3 days of life, and were most prominent in infants with IVH and WMI. These two studies with seizure incidence of 43-48% are very high compared to some other aEEG studies, and all EEG studies. This might be due to misinterpretation such as identifying biological and external artefacts, high-amplitude rhythmical slow activity, differing GA and state changes as seizures. Certain artefacts can raise the aEEG baseline, mimicking seizures, such as movement, muscle, respiration, and hiccup artefact. Furthermore, a recent study by Weeke et al. showed that seizures can often be misdiagnosed due to the familiar normal rhythmic activity on preterm EEG (328). These normal rhythmic EEG patterns do not evolve in amplitude, frequency of morphology and are common during the first 72 hours after birth. Therefore, Weeke et al. suggested that these waveforms could have been considered as seizures due to the similar morphology and lack of multichannel application could have limited their ability to confirm the episodes.

Summary of preterm aEEG/EEG seizure incidence

Identification of seizures varies significantly between monitoring approaches. In terms of seizure incidence, large inconsistencies exist between studies, with results ranging from 0.9% - 48% across all studies. A clear difference is noticeable, with aEEG studies reporting higher seizure frequencies compared to EEG studies (6.6 – 48% vs 0.4 – 3%, respectively). A possible reason for the lack of accuracy when using aEEG could be due to the limited electrode application and trending displays. It is important to ensure that aEEG review does not only involve interpretation of the trend, but also review the raw EEG trace of those channels. The multichannel EEG is the gold standard allowing even distribution of electrodes over multiple brain areas, whereas certain brain areas are not covered by the aEEG. Therefore, depending on the methodology, such as length of recording, the EEG studies may provide more reliable results.

Methodological differences are evident between studies, including monitoring duration and start time of recording. One clear advantage from the aEEG studies is that application is easier and quicker and can often be applied by nursing staff, with most recordings starting within the first 72 hours of life, which is when seizures most commonly occur (305, 314, 315, 318). Monitoring duration from the majority of aEEG studies varies between 1 -174 hours, while the majority of EEG studies only involving 1- or 2-hour recordings. Longer recordings are likely to be more effective in detecting seizures, particularly when seizures are occurring infrequently, in addition to monitoring electrographic changes during brain development.

This highlights the need to improve the EEG application approach and the knowledge of seizure frequency in preterm infants. A clear difference in opinion is evident regarding seizure incidence in preterm infants, which is of concern as it suggests that potentially, either true seizures may be being missed, or the converse that non-seizures may be being inappropriately treated. Thus, further research using long-duration multichannel EEG is required to accurately report seizure incidence in preterm infants. As automated algorithms are being designed for full term seizure detection, perhaps a dedicated algorithm for preterm EEG would be valuable. A dedicated seizure detection algorithm is probably needed for preterm infants as preterm seizures are different to seizures in full term infants, especially in duration and burden (329).

1.12. Neurodevelopmental Outcome

Preterm infants are at risk of adverse neurodevelopmental outcome. There are numerous types of outcome assessments, namely; the Griffiths Mental Developmental Scales, the Tsumori-Inage and Kyoto Scales, the Bruner-Lezine test, the Ages and Stages Questionnaire, the Scheffzek test, the Denver II Developmental Screening Test, the Peabody Developmental Motor Scales and the Bayley Scales of Infant and Toddler Development. The Griffiths Mental Developmental Scale assesses the subscales of locomotor, personal/social, hearing and language, eye-hand co-ordination and performance. It is a well-established scale which has been reported as a good predictor of outcome at 1/2 years of age, with certain limitations such a not detecting isolated hemiplegias (330). The Ages and Stages Questionnaire is a parental questionnaire that screens for developmental delay by establishing which activities the child is able to complete. This introduces bias into the equation, whilst it also focuses on personal-social ability (self-help skills and interaction with others) rather than social-emotional ability (identifying the major social-emotional milestones). However, it is quick and easy and studies report high sensitivities and specificities, suggesting that the questionnaire is valid for screening preterm and full-term infants (331, 332). The Denver II Developmental Screening Test considers the subscales of social, fine motor function, language and gross motor function. This second version of the test was developed due to the lack of evidence regarding its accuracy. Although the sensitivity of the test is high, it has been reported that the specificity is low at 43%, questioning it's reliability for predicting outcome (333). The Peabody Developmental Motor Scales is purely a scale for motor development which sees 6 different subscales of reflexes, stationary, locomotion, object manipulation, grasping and visual-motor integration. Although the reports show high test-retest and inter-rater reliability, it is believed that the test is not sensitive enough to distinguish fine motor function (334).

In Cork University Maternity Hospital, preterm infants are routinely invited to clinics to review their progress. The final review is at 2 years of age, where different aspects of development are reviewed, using the Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III) (335).

1.12.1. Bayley Scales of Infant and Toddler Development -III

This assessment is performed on a one-to-one basis with an individual that has been trained in the test, such as a child psychologist, who assesses the mental and motor development of the child between 1 and 42 months of age. This is a test that's based on up to date standardized and normative data (score of 100), allowing estimation of developmental delay. This allows early professional intervention with the intention of providing support for developmental improvement (336). This third edition of the test targets five developmental domains: cognitive, language, motor, social-emotional and adaptive behaviour. The cognitive, language and motor are regarded as the main domains in terms of usefulness in research.

Cognitive

This scale is comprised of 91 items, with numerous items taken from the Mental scale section of the second edition. These items assess sensorimotor development, exploration and manipulation, object relatedness, concept formation and memory (337).

Language

The language scale is divided into two subsets: receptive and expressive communication. The receptive communication subset targets the child's auditory acuity in addition to their understanding and response to verbal instruction. The child's ability to communicate with people, vocalise and name pictures and objects is assessed in the expressive communication subset (335).

Motor

Two subsets are also involved in the motor scale: fine motor and gross motor. The fine motor subset concentrates on eye movements, perceptual-motor integration, motor planning and motor speed. Movement of limbs and torso are focused upon in the gross motor subset (335).

Social-emotional

A revision from the Bayley-II test was to include this new domain which is a questionnaire completed by the child primary caregiver e.g. the parent. This questionnaire attempts to identify the major social-emotional milestones of the child (337).

Adaptive Behaviour

This domain was also introduced into this third version of the test, and is also a questionnaire for the caregiver. Assessment of the daily functional skills is aimed at determining levels of behaviour such as health and safety and self-care (335).

Comparisons between the assessments have previously been reported. One study that compared the performance of the Bayley scale assessment and the Griffiths

Mental Scale. Composite and quotient scores were calculated, and Bayley scale gave a better measurement of development (338), while another study suggested that the Griffiths Mental Scale overestimated neurodevelopment impairment compared to Bayley scale assessment III (339). A comparison with the Ages and Stages Questionnaire raised concerns about the performance of the questionnaire, with poor sensitivities and specificities suggesting that it is not a sufficient substitute (340).

Bayley Scales of Infant and Toddler Development is well-regarded tool which is validated in the UK and Ireland, available to use in a wide range of clinical conditions, and also has materials that keep the children engaged. The tool is used in numerous studies, especially in those that research the practicality of EEG in neonates. These studies differ in terms of how the Bayley summarised neurodevelopmental delay, with some studies opting to define a delay as less than 15 points (1 standard deviation) from the mean in any of the three domains (310, 311, 314, 341), some by 30 points (2 standard deviation) from the mean in any of the domains; < a score of 70 (305, 315, 326), whilst one study scored by 1 standard deviation in all three domains, or alternatively by 2 standard deviation in one domain (342). One study assessed the suitability of these cut offs, reporting that cognitive and language scores of <85 or combined Bayley III scores of <80 are preferable, however this required further validation (343). In this thesis, a Bayley III assessment score of <85, in any of the three domains, will be used to test for neurodevelopmental delay for infants at two years of age, based on the popularity of this approach.

1.13. Summary

Preterm infants are at increased risk of neurodevelopmental problems. The aetiology is multifactorial, but brain injury in the form of IVH and/or PVL are two of the leading causes. Many other factors can impact brain growth and development at this crucial time in maturation including episodes of sepsis, NEC and BPD. Significant advances over the last three decades have seen a reduction in the incidence of many of these conditions, with a resultant improvement in developmental outcome. However, IVH and PVL still remain significant problems, especially in the more immature infants who remain at greatest risk of adverse short and long-term problems. Whilst monitoring neurological well-being in preterm infants in the NICU remains challenging, the potential benefits may be significant and neurophysiological brain monitoring is now growing in popularity.

The current understanding of preterm EEG is limited and its prognostic value is unclear. The most common clinical tool to assess premature brain activity continues to be the aEEG due to its ease of use, however multichannel EEG remains the gold standard. There are many obstacles which complicate EEG application, the most obvious being infant handling and another being that it is time consuming. Recording multichannel EEG is challenging in this cohort of vulnerable infants, however the aEEG is very limited and can lead to misinterpretation, therefore it is important to discover whether conventional EEG should be used as a clinical tool at this young age. If there is a place for it clinically, a simplified application process is needed to benefit untrained clinical staff.

Current literature does suggest that there is a place for EEG as a prognostic tool, however more long-term, multichannel-EEG studies are required. Seizure frequency in preterm infants is unclear with results varying from 0.9% - 48%, between EEG and aEEG studies. Limited long-term, multichannel-EEG studies highlights the need for continued research in this area.

1.14. Aims and Scope of Thesis

The specific aims of the thesis is to:-

1) assess the potential of conventional EEG, recorded within days after birth, and multimodal analysis for predicting long-term adverse **(chapter 3)**;
2) describe the frequency and characteristics of seizures in preterm infants in the early postnatal period **(chapter 4)**;
3) develop an age-specific EEG assessment system **(chapter 5)**;

4) investigate the EEG similarities and effect of maturation in identical and non-identical twins **(chapter 6)**;
5) assess the ability of serial multichannel EEG for the prediction of long-term adverse outcome **(chapter 7)**.

Retrospective EEG recordings were used in Cohort 1 however they were recorded in the first few days after birth only and not repeated at later stages in neonatal course. The prospective study (cohort 2) aimed to record the continuous multichannel video-EEG as early as possible, in addition to shorter recordings at 32 weeks and 35 weeks. This ensured enough data was collected to investigate evolution of the EEG without impacting the well-being of the infants. Preterm infants require minimal handling, therefore the amount of electrodes used and amount of recordings performed were discussed and agreed with the Consultant Neonatologists.

Chapter 2. Methodology

Part published as:

"Overcoming the practical challenges of electroencephalography for very preterm infants in the neonatal intensive care unit."

Lloyd R, Goulding R, Filan PM, Boylan GB. Acta Paediatrica 2015;104:152-7.

(Published February 2015).

This chapter outlines the methods used to recruit preterm infants in Cork University Maternity Hospital, the approach for EEG monitoring and analysis, and the procedure for performing neurodevelopmental outcome assessment. The following chapters will refer to this chapter for the general methodology used, with more precise methodology specified in subsequent chapters.

2.1. Subjects & Settings

This is an observational study concentrating on EEG analysis of preterm infants (<32 weeks gestation). Recruitment took place in the NICU of Cork University Maternity Hospital, a large tertiary maternity hospital of 8-9,000 deliveries per annum, of which approximately 80 – 100 are preterm infants below 32 weeks.

Cohort 1 – Retrospective cohort

Any infant above 24 weeks GA and below 32 weeks GA was recruited over a 2-year period between April 2009 and March 2011. In addition, any infant with clinical concern of seizure, on NIRS monitoring, with Apgar score ≤ 6 at 5 minutes, first blood gas pH ≤7.1 or first base deficit ≥16 were recruited for EEG monitoring.

Cohort 2 – Prospective cohort

Preterm infants less than 32weeks GA were recruited over a 2-year period between March 2013 and April 2014. The exclusion criteria excluded infants with known congenital anomalies, which were deemed likely to affect future long-term development were excluded.

Figure 2-1 illustrates how cohort 1 and 2 were involved in the succeeding chapters.

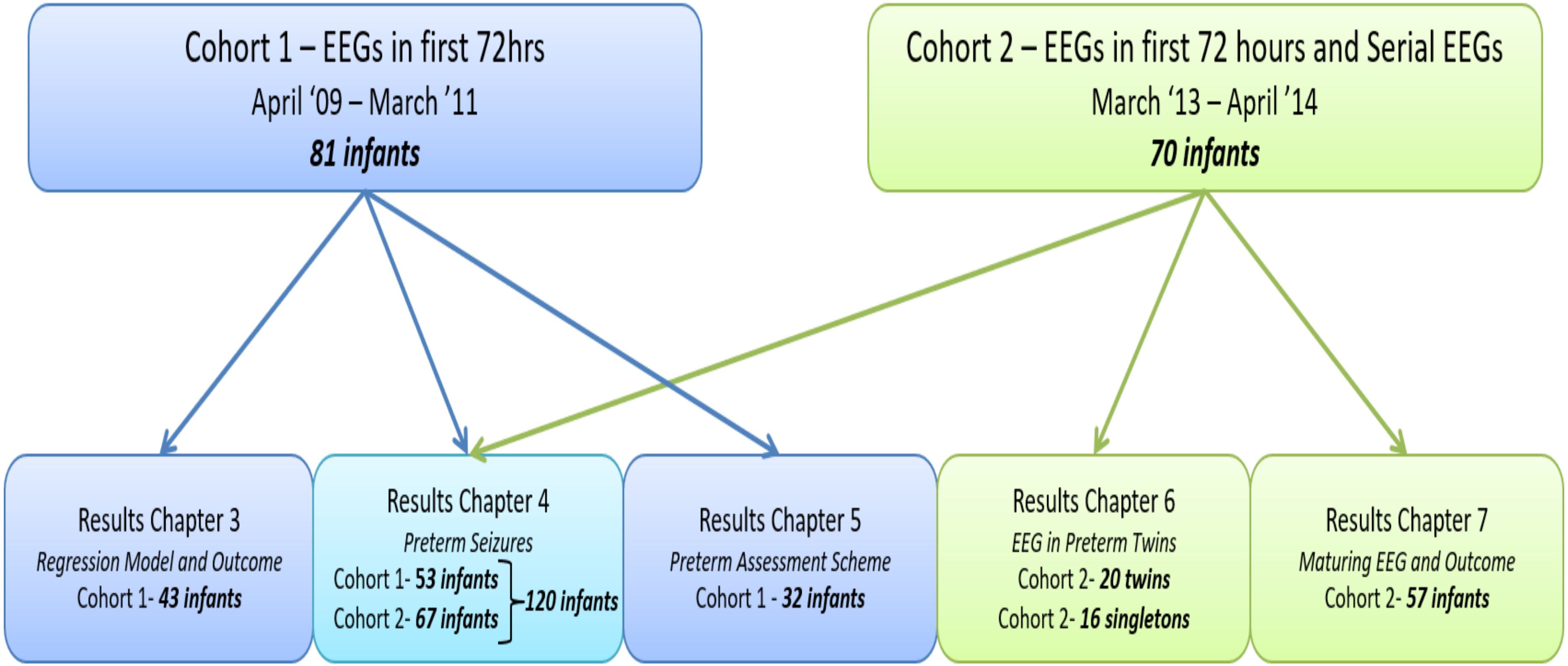


Figure 2-1 Illustration of which cohorts were studied in each result chapter.

2.2. Ethical approval and study protocol

The prospective study protocol was reviewed and approved by Cork Research Ethics Committee (CREC) on the 20th of February 2013 (Appendix A). Approval required a study protocol, (Appendix B), parent information leaflet (Appendix C) and consent form (Appendix D). Standard operating procedures (SOP) provided guidelines for the study, such as applying EEG electrodes and obtaining informed consent. SOP and Good Clinical Practice training was provided to every member of the research staff involved in this current study. A list of the co-investigators is evident in appendix B.

The retrospective study protocol was previously reviewed and approved by CREC on the 7th of October 2008. All study personnel implemented the clinical investigation with full respect and compliance of the legal and ethical European and institutional requirements and codes of practices. All data was saved and pseudo- anonymised and kept within the department. Data and results were stored on the university server and an encrypted hard-drive and accessed through a secure university computer.

Written informed parental consent from both parents (if possible) or guardian was obtained either antenatally or postnatally, depending on the individual circumstances of each eligible infant. Initially the parents were approached and asked if they would like to discuss the ongoing study. The Information leaflet was presented and discussed, before any further questions were received. Following a detailed explanation, the leaflet was left with the parents to read and discuss in private for an appropriate amount of time. Questions and clarifications were answered before consent was later obtained. Consent was undertaken by the co-investigators, evident in appendix D.

2.3. EEG data acquisition

Multichannel EEG monitoring was performed for both cohorts. Monitoring began as soon as possible after birth and continued for up to 72 hours after birth, approximately. However, depending on the stability of the infant, monitoring may have continued past 72 hours if the clinical staff deemed appropriate. EEG electrode application in this population was difficult and challenging, due to the small head size and the limited space within a humidified incubator. These infants frequently required respiratory support and head caps were often used to secure respiratory devices, such as a continuous positive airway pressure (CPAP) hat. Strict infection control and hand hygiene guidelines were adhered to and standardising the technique for electrode application, without affecting the quality of EEG recordings, was essential. The methods for EEG application and recording in the NICU has been published to Acta Paediatrica in 2015 (344).

2.4. EEG electrode application procedure

From the review of currently available methods for neonatal EEG recording, we concluded that an optimal method for EEG monitoring in very and extremely preterm infants is not available. We therefore developed a technique using the prepackaged, disposable, sterile, flat-surfaced electrodes. The EEG application procedure was timed in 10 cases and the average time required to apply the electrodes in the incubator was 12 minutes.

EEG electrodes were applied by trained EEG technologists or medical personnel. The method was easily adopted by staff in the NICU, following two or three training sessions. Prior to the application of EEG electrodes, close consultation with NICU personnel was complete to ascertain policies and procedures that must be adhered to while in the NICU environment and to ensure that the health, safety and well-being of the preterm infant is not compromised in any way during handling and application. The step-by-step procedure is outlined below.

Adequate preparation is paramount to the success and efficiency of the procedure. Strict hand hygiene is mandatory within the NICU environment, therefore hands must be washed in line with the hand hygiene protocol prior to handling of any EEG materials and surfaces must be cleaned before use in accordance with local guidelines (345). The materials required are as follows:

- Disposable, sterile, flat-surfaced electrodes with the ideal dimension of 15mm x 20mm, with a measuring area of 263mm^2. Due to the small head size, the surface area of the electrodes needs to be small. Ambu Neuroline 700 Single Patient Surface Electrodes were used for our technique. The estimated cost of a single pack of 12 electrodes is €8 plus value added tax.
- EEG Machine. Any EEG system can be used for this procedure. In our centre, three machines were used with the signal sampled at 256Hz (NicoletOne, ICU Monitor,

NeuroCare, Carefusion; Nihon Koden, EEG-1200, Neurofax and Moberg ICU Solutions, CNS-200 EEG and Multimodal Monitor).(Figure 2-2)

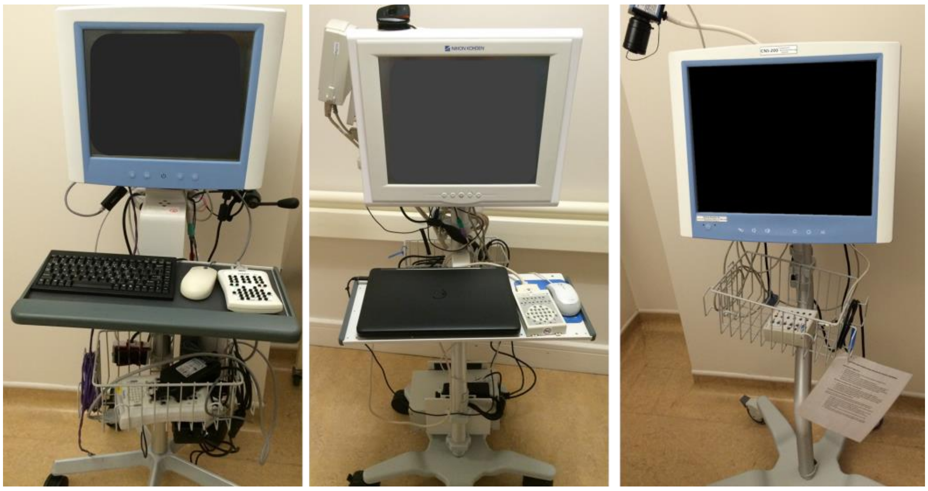


***Figure 2-2* Three EEG machines used for recording the neonatal EEGs – (left to right) NicoletOne, Nihon Koden and Moberg ICU Solutions.**

- Coloured pencils. Colour coded, pre-labelling of the electrodes for each hemisphere minimises possible human error during electrode application. In addition, pre-labelling helps with a more systematic approach to applying electrodes, and ensures the application is less time consuming and more efficient.
- Adhesive medical tape (Mefix). Electrode contact weakens in the high humidity of the incubator, therefore securing the electrode with a small square piece of tape minimises loss of contact. Ten20 paste can be applied to the tape, if required, to enhance adhesion.
- Stockinette. EEG electrode cables are contained together, in an orderly fashion, within stockinette or other suitable tubing.
- Skin prep gel. This improves conductivity.
- Sterile tongue depressor. Facilitates clean transfer of electrode paste.
- Sterile cotton buds. Used in conjunction with the skin prep gel to minimise the impedance of skin.
- Sterile galipot. Provides a sterile container for the skin prep gel and Ten20 paste.
- CPAP hat. Provides electrode security.
- Disposable gloves.

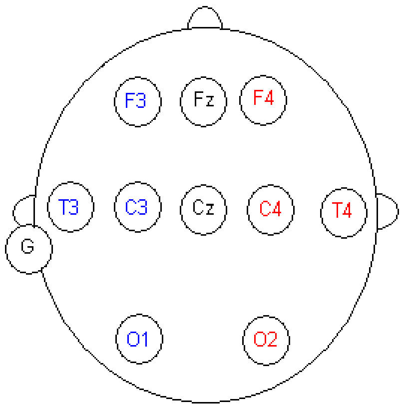


Figure 2-3 International 10/20 system modified for neonates was followed for application.

The materials required should be organised prior to electrode preparation. The international 10/20 system (Figure 2-3) of EEG electrode placement, modified for infants, should be followed, as described in the American Clinical Neurophysiology Society Guidelines (171). We prefer to use the F3/F4 electrode positions instead of the standardised prefrontal positions (Fp1 & Fp2), located more anteriorly, as we have found that the F3/F4 electrodes are less susceptible to falling off in the humid incubator environment. In addition, we routinely use near-infrared spectroscopy monitoring, which requires a sensor over the frontal region. It should be appreciated that by using the F4/F3 electrode, the amplitude of the EEG in a longitudinal bipolar montage (between F3-C3 and F4-C4) will be reduced, in comparison to the amplitudes recorded over C3-O1 and C4-O2 channels, due to unequal inter-electrode distances. However, whichever electrodes are used to record the EEG, it is imperative to ensure that inter-electrode distances between hemispheres are equal.

2.4.1. Electrode preparation

- Prepare and organise the required equipment on a clean work tray placed on a clean trolley. The trolley and work tray must be cleaned with disinfectant wipes or a similar alternative, in accordance with local guidelines, before proceeding. All materials required will be placed in the clean work tray, once organised.

- Allocate an electrode set to the right cerebral hemisphere and label F4, C4, T4 and O2. Allocate another to the left hemisphere labelled F3, C3, T3 and O1. Label three more electrodes as Reference, Ground and Cz (Figure 2-4).

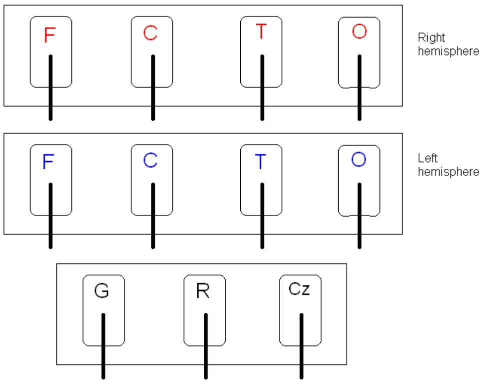


Figure 2-4 Labelling the positions on the electrode surfaces eased the application process.

- Cut some Mefix tape, label and apply to each corresponding electrode plug (Figure 2-5).

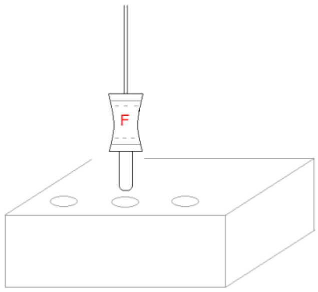


Figure 2-5 Labelled electrode socket plugged into headbox

- Cut an appropriate length of stockinette and feed the electrodes through it to keep them in order (Figure 2-6). This reduces electrode entanglement and 50Hz artefact interference.

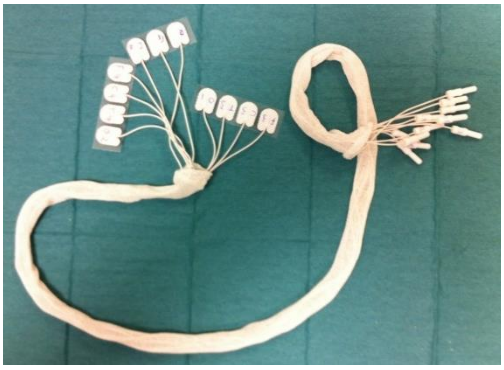


Figure 2-6 Pre-labelled electrodes positioned inside the stockinette

- Insert the electrode plugs in their designated sockets in the EEG amplifier.
- Cut small pieces of Mefix tape to help secure the electrodes (Figure 2-7).

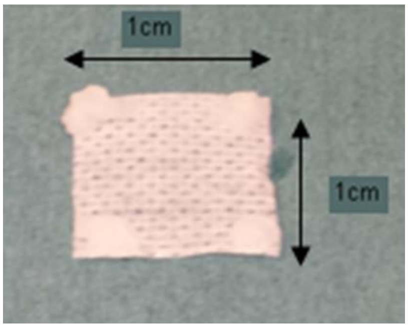


Figure 2-7 Cover electrode with tape and paste

2.4.2. Electrode Application

- Wash hands again, as per dedicated hand hygiene protocols, and open the incubator portholes for access. Using gloves is advisable.
- In our NICU, infants on CPAP have a hat in place to secure the CPAP mask. Cut the CPAP hat down the frontal midline region and lay it open. The CPAP mask needs to be held in place while electrode application is ongoing, so an assistant is required at this stage. In the intubated and ventilated infant a CPAP hat is still applied, as it helps to protect and maintain the position of electrodes.
- Part the hair at the electrode site, and gently abrade the skin three to four times using a sterile cotton bud and a skin preparation gel such as Nuprep.
- Apply the electrodes to one side of the head and then the other, to reduce head turning and disruption to the infant (Figure 2-8).

Figure 2-8 Electrode positions on right hemisphere covered with tape.

- Position the electrodes so that all leads are directed towards the vertex (Figure 2-8). From here, they enter a stockinette, which helps minimise 50 Hertz interference.
- Cover each electrode with the pre-cut Mefix tape, to improve stability. This also prevents electrode bridging between adjacent electrodes (346).
- Re-secure the CPAP hat using the Velcro strap and additional tape (Figure 2-9).

Figure 2-9 CPAP hat closed ready for recording

- Check all impedances before finishing the procedure. It is important that all electrodes have equal and low impedance, which tends to improve over time.
- At the end of the recording, carefully remove the electrodes one by one. Slowly peel the tape from the corner in the direction of which the hair lies, to prevent pulling.

2.4.3. Data Storage and Protection

Recorded data was collected and immediately pseudo-anonymised, to ensure the safe storage of confidential data, in accordance with the guidelines for Good Clinical Practice. The start and duration time of the EEG monitoring were documented, whilst the recoding type i.e. initial or repeat recording was also noted. The neonatal medical notes provided the demographic information at the time of recruitment. The information collected during the infants stay in the NICU can be seen in the table 2-1:-

General Info	
Gender Gestational Age (weeks) Date of Birth Time of Birth Estimated Date of Delivery Birth Weight (g) Apgar 1 & 5	Infant demographics and clinical details were collected from the electronic database discharge summary document (Badger neonatal system, Badger 2003) or the medical notes. Collecting from both documents confirmed accurate findings.
Delivery	
Type: Information from medical notes and Badger system. inc. Spontaneous vaginal delivery; Emergency cesarean section; Planned caesarean section. Resuscitation: Information from medical notes and Badger system. inc. Stimulation, PMA, O_2, Suction, Bag & Mask IPPV, Intubation, stimulated intermittent positive pressure ventilation, surfactant. CRIB II: Information from Badger system or can calculate from gender, gestational age (GA), Birth weight (BW), admission temperature, and the base deficit in the first blood sample	
Maternal Info	
Age Contact Information Smoking Other	Information from medical notes.
Information from medical notes. inc. PROM, ovarian cysts, previous deliveries or miscarriages, urinary tract infections, preeclampsia, cardiotocography and doppler results, diabetes, hyper/hypotension.	
Infant's Clinical Course	
Neurological (IVH/cPVL) – Information from the picture archiving and communication system (IMPAX) Infections (Sepsis) – Information from Citrix 4.5 and iLAB	
Respiration support (BDP/CLD) Cardio Gastro Intestinal (NEC) Ophthalmology (ROP) Jaundice Anaemia Surgery Discharge Weight	Information from medical notes and Badger system
Medication – Information from drug charts in the medical notes	

TABLE 2-1 COLLECTED DATA DURING THE INFANTS STAY IN THE NICU

2.5. EEG Visual analysis

The EEG was analysed to grade the normality of the preterm EEG and identify any electrographic seizure activity. The whole EEG recording was reviewed for seizure activity, whilst epochs were pruned at specific time-points for grading purposes (with the length depending on required study aim). This was performed by a clinical physiologist (RL[2]) and depending on the study a second examiner was involved for revision (EP[3]). An experienced neonatal physiologist (GB[4]) also reviewed the recordings for a second opinion. More details for analysis will follow in the individual chapters, due to the different approaches undertaken.

2.5.1. Artefact Identification

During analysis, it was important to recognise different artefact types. Presence of artefacts during monitoring will overshadow the EEG and deteriorate the quality of recording. Therefore, epochs with minimal artefact should be selected for analysis, allowing brain function to be accurately investigated. The following figure (Figure) are examples of artefacts witnessed during EEG recordings in preterm infants in a NICU environment.

[2] Rhodri Lloyd
[3] Elena Pavlidis
[4] Geraldine Boylan

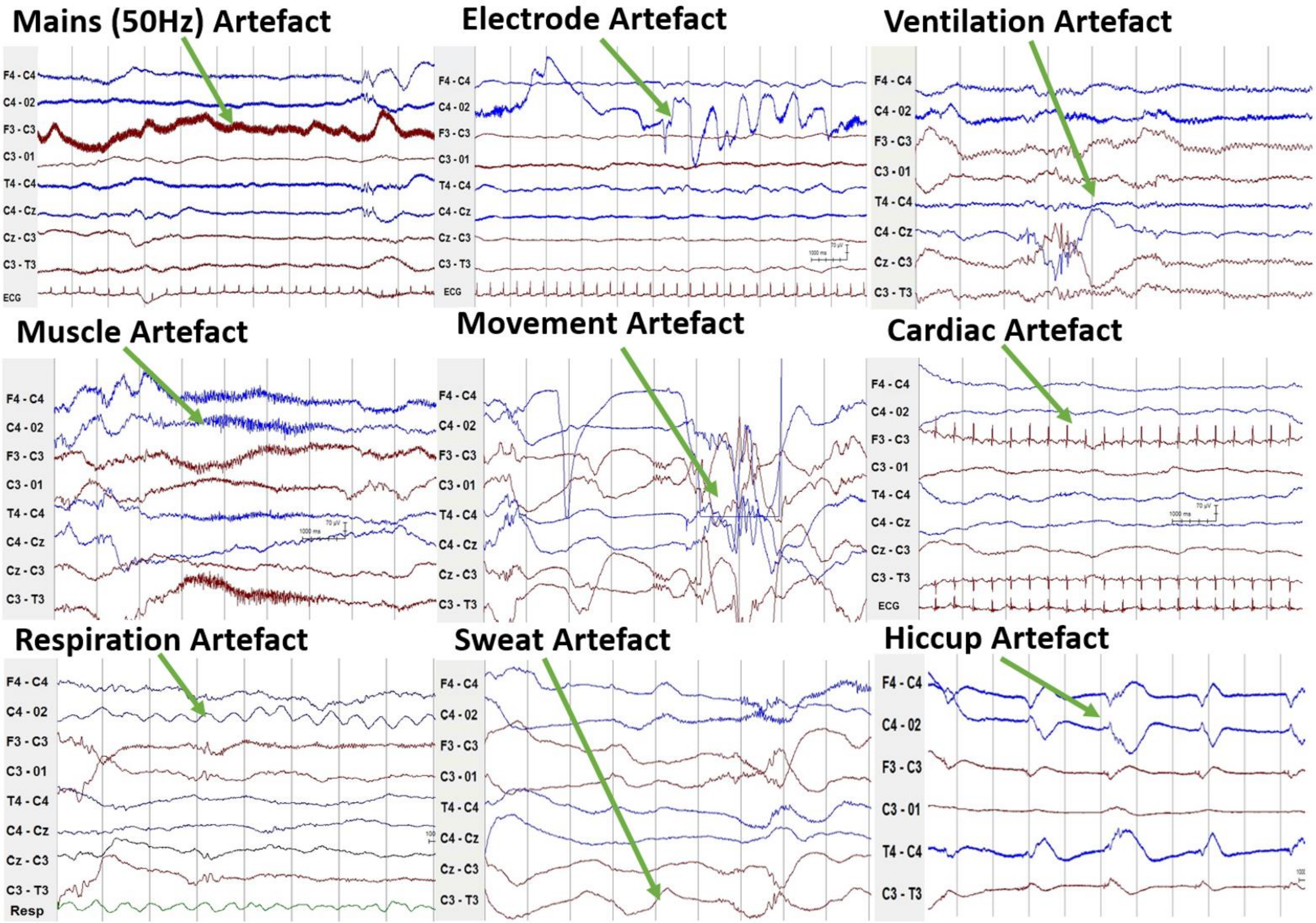


Figure 2-10 Examples of EEG artefacts witnessed in neonatal EEG recordings

2.6. Neurological Developmental Analysis – Bayley's (III)

Neurodevelopmental outcome was assessed at 2 years corrected age in all surviving infants using the Bayley Scales of Infant Development III. Assessment for the retrospective cohort was performed by a specialist neonatal physiotherapist (Anne Marie Cronin) whilst the prospective cohort was performed by a research psychologist (Emma Hennessy). Both were trained to implement the test and had experience in practice.

This assessment measures the child's cognitive, language, motor, socio-emotional and adaptive behaviour development and provides 5 subscale scores. For research purposes, 3 subscales scores were obtained (cognitive, language and motor). An abnormal outcome was defined as any of the 3 subscales being below one standard deviation from the mean; thus for the standardized scores, a value of less than 85 in any of the 3 subscales was deemed abnormal (310). Conversely, a normal outcome was defined as every subscale being 85 or above. Infants who died during their time in the NICU were also allocated to the abnormal outcome group.

2.7. Statistical analysis

The software used to undertake statistical analysis was IBM SPSS software version 21 and SAS 9.3 (SAS Institute Inc., Cary, NC, USA) or Stata 13.0 (StataCorp LP, College Station, TX, USA. Guidance was provided by Dr Vicki Livingstone (statistician), who confirmed the accuracy of the statistical analysis results. Further details regarding statistical testing for each chapter is outlined in the relevant chapters.

Chapter 3. Predicting two-year outcome in preterm infants using early multimodal physiological monitoring

Part published:

"Predicting 2-y outcome in preterm infants using early multimodal physiological monitoring."

Lloyd RO, O'Toole JM, Livingstone V, Hutch WD, Pavlidis E, Cronin AM, Dempsey EM, Filan PM, Boylan GB. Pediatric research 2016;80(3):382-8. (Published September 2016).

3.1. Introduction

Of the 15 million premature births worldwide each year, one to three million infants will die, approximately 10 - 12% will develop CP and a further 19% will develop motor or cognitive problems (6, 347). Accurate and early prediction of neurodevelopmental outcome in the preterm infant provides important clinical information that can be used to guide early intervention, assist clinical management, and ensure appropriate long-term needs are identified. Predicting outcome at 2 years or more, in the first few days after birth is ambitious however, as preterm infants are vulnerable to brain injury during their entire stay in the NICU (348).

Studies have attempted to predict short term outcome, within the NICU period. Early clinical information, including Apgar scores, gender, BW, GA (39, 349-351) and illness severity scores, such as SNAP-II and SNAPPE-II have been used to predict short term outcome (352). Multivariate models including clinical risk factors such as GA, BW and gender, have shown promise for predicting long term outcome (353, 354). Analysis of multiple risk factors combined in a multivariate model can improve outcome prediction (351). Saria *et al.* showed that a combination of quantitative features of early physiological measurements, including heart rate (HR), respiratory rate (RR), and peripheral oxygen saturation (SpO_2), could predict short-term outcome with a high level of accuracy (sensitivity of 86% and specificity of 96%) (355). The absence of a reliable measure of neurological function, however, may limit the ability of these approaches to predict neurodevelopment in the longer term, beyond the early intensive care stage. Preterm infants can show physiological instabilities, such as low SpO_2 levels and decreased variability in heart rate. Arterial SpO_2 measures the amount of oxygenated haemoglobin in the blood. Oxygen desaturation relates to a decrease amount of oxygen in the blood. A systematic review reported that SpO_2 values of approximately 85 – 95% should be targeted for preterm infants (356). Heart rate variability is the variation over time in the interval between heartbeats, providing assessment of the functional state of the autonomic nervous system.

Previous studies have shown that analysis of early measurements of EEG can predict long-term neurodevelopmental outcome, with specificity and sensitivity ranging from 88% - 96%

and 25% - 83%, respectively (293, 307, 321). Other studies have shown that the aEEG can predict long-term outcome, with specificity ranging from 73% - 89% and sensitivity ranging from 56% - 87% (267, 315, 341). To date, however, no standardised method for the accurate prediction of long-term outcome in very preterm infants has been successfully translated into clinical practice.

The aim of this chapter was to determine if multimodal physiological monitoring including EEG, recorded in the first day of life, combined with demographic risk factors such as BW and GA, can predict outcome status at 2 years of age in very preterm infants. The multimodal model combines EEG grading with quantitative features of routinely-available physiological signals, namely SpO_2 and HR (355). A clinical course score, which represents a good estimate of long-term outcome from clinical history of the intensive care period, is used to compare performance of this multimodal approach.

3.2. Methods

3.2.1. Participants

This was a retrospective, observational study performed in the NICU of Cork University Maternity Hospital. As seen in Figure 2-1 in the methodology chapter, the eligible infants were all preterm infants from cohort 1 (<32 weeks gestation) born between April 2009 and March 2011. Preterm infants were included in the study if they had continuous multichannel EEG monitoring with simultaneous registration of SpO_2 and HR, and neurodevelopmental assessment at 2 years. Ethical approval was granted by the Clinical Research Ethics Committee of the Cork Teaching Hospitals, Ireland. Written informed parental consent was obtained.

3.2.2. Physiological Recordings: EEG, SpO_2 and HR

The NicoletOne EEG system (CareFusion Co., San Diego, USA) was the only machine used to record continuous video-EEG. All EEG recordings were initiated within 24 hours of birth. EEG application procedure for this chapter is explained in chapter 2, with the only difference being that silver-silver chloride electrodes were used. A Philips IntelliVue MP70 monitor (Philips Medical System, BG Eindhoven, The Netherlands) was connected to the NicoletOne EEG system, which consequently synchronised the SpO_2 and HR with the EEG waveforms (Figure 3-1). Monitoring continued for up to 72 hours after birth, depending on the stability of the infants.

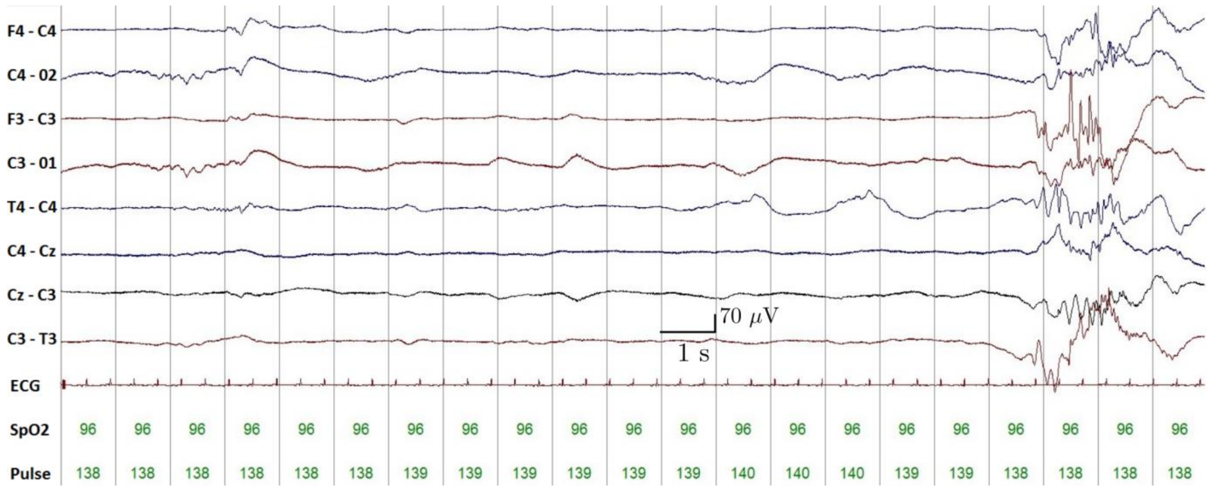


Figure 3-1 Example of multimodal signals – Recording displays the raw EEG, SpO_2 and HR channels. EEG recording of male 26+0 week GA at 9 hours of age.

3.2.3. EEG Data Collection

The EEG signal was sampled at 256 Hz, and the SpO_2 and HR were sampled at 1 Hz. The EEG recordings were visually analysed for quality and, if this was poor, the infants were excluded.

The entire EEG recording in each infant was assessed for seizure activity, state change and maturational features such as delta brushes, occipital delta waves and temporal sharp waves. The EEGs were graded by two clinical physiologists (RL and GB[5]) who were blinded to all clinical information except for GA, administration of morphine or phenobarbitone and time of EEG recording post-delivery. The EEG recordings were scored based on the grading system described by Watanabe et al (194), which differentiated acute abnormalities (ASA) from those

[5] Rhodri Lloyd and Geraldine Boylan

of the chronic stage (CSA). ASA were defined as suppressed background activity, decreased continuity, low amplitude and attenuated fast-wave background. CSA included dysmature patterns and disorganised patterns, such as abnormal delta waveforms, sharp waves and abnormal delta brushes. EEGs can be classed as mild, moderate or severe (194, 294). EEGs were reviewed and consensus was achieved for each recording. Inter-rater agreement was assessed using Cohen's kappa coefficient.

One-hour epochs of EEG at 12 and 24 hours of age, were then extracted from each recording for grading and multimodal analysis. These specific time-points were selected for analysis due to the fact that they represented the most consistent time points when multimodal data was available for the entire cohort. Most recordings included both time-points, but some were missing due to late application, instability of the infant or poor quality of the EEG recording at that time period. When both time-points were available, the EEG grades were combined and the most abnormal grade was selected.

3.2.4. Additional data collection

One-hour epochs of HR and SpO_2 were extracted at the two time-points, 12 and 24 hours. Two features were used to summarise SpO_2 for the one hour epochs: mean SpO_2 and percentage of time <85%, which represents the degree of hypoxia (357, 358). Four features summarised the HR signal over the one hour epochs: mean, standard deviation, skewness, and kurtosis (359). The standard deviation represents the variability of the HR segment; skewness represents the tendency of the HR signal to include large-amplitude transients in either the positive or negative directions which relate to accelerations and decelerations of the HR (360); and kurtosis quantifies the deviation of the HR signal from a Gaussian process, often the result of high-amplitude transients. These higher-order statistics were included as previous studies relate short-term outcome to changes in the skewness and kurtosis (361). When available, the mean values of the features over both time points were used for subsequent analysis. Clinical and demographic characteristics were also collected.

3.2.5. Assessment of Clinical Course

Infant demographics and clinical details were collected from the electronic database discharge summary document and the medical notes. Blinded to infant identity and physiological data, two consultant neonatologists (PF and ED[6]) reviewed the discharge summary documents and medical notes for all infants. Each infant was classified as either at high or low risk of later morbidity based on their clinical course score. Infants were allocated as having a 'complicated' clinical course if they suffered from any of 5 major complications during their time in the NICU (Table 3-1). When grades differed between reviewers, a consensus was reached by discussion.

5 Major Complications
• Grade III/IV intraventricular haemorrhage (IVH) or cystic periventricular leukomalacia (cPVL) • Bronchopulmonary dysplasia (BPD) as defined by oxygen dependency at 36 weeks post menstrual age (PMA) • Necrotizing enterocolitis (NEC) Bells stage 2b or greater • Infection – positive blood culture with abnormal inflammatory markers, white cell count (WCC) or C-reactive protein levels (CRP) • Retinopathy of prematurity (ROP) Stage 2 or greater

TABLE 3-1 DEFINITIONS FOR MAJOR NEONATAL COMPLICATIONS.

3.2.6. Two-year outcome assessment

Neurodevelopmental outcome was assessed at 2 years corrected age in all surviving infants using the Bayley Scales of Infant Development III. In this chapter the test was performed by a specialist neonatal physiotherapist (AMC[7]). The method of this assessment is previously described in chapter 2, where 3 subscale scores from the child's motor, cognitive and

[6] Dr Peter Filan and Prof Eugene Dempsey
[7] Ann-Marie Cronin

language development is assessed. An abnormal outcome was defined as any of the 3 subscales being below one standard deviation from the mean; thus for the standardized scores, a value of less than 85 in any of the 3 subscales was deemed abnormal (310). Conversely, a normal outcome was defined as every subscale being 85 or above. Infants who died were also allocated to the abnormal outcome group. Figure 3-2 illustrates the infant's course in the NICU and up to 2 years of age.

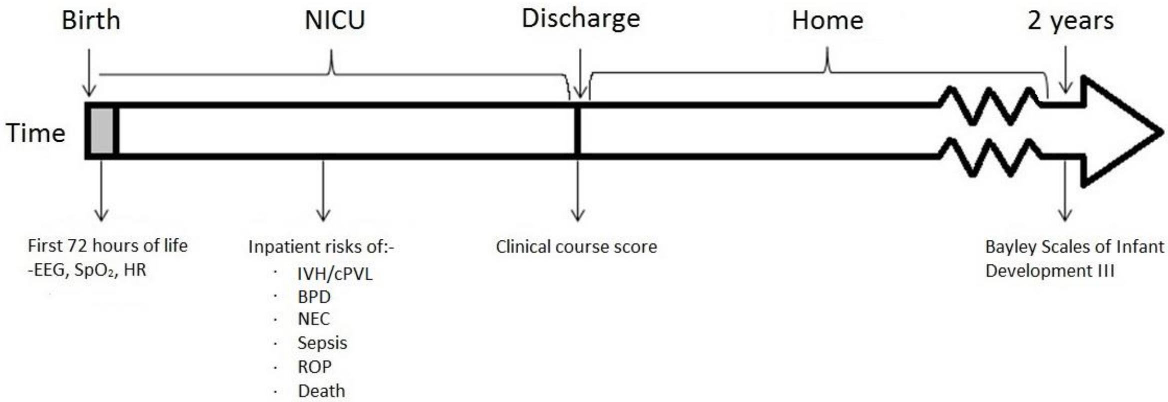


Figure 3-2 Timeline of infant's stay in the NICU through to the neurodevelopmental follow up at 2 years of age. IVH, intraventricular hemorrhage; cPVL, cystic periventricular leukomalacia; BPD, bronchopulmonary dysplasia; NEC, necrotizing enterocolitis; ROP, retinopathy of prematurity

3.2.7. Statistical Analysis

For statistical analysis, EEG grades were grouped into two categories: 1 = normal or mildly abnormal and 2= moderately or severe abnormal (362). Continuous variables were described using mean (standard deviation, SD) and median (inter-quartile range, IQR) where appropriate and categorical variables described using number (percentage). The ability of each physiological feature to predict either normal or abnormal outcome was assessed using the Mann-Whitney U-test (continuous data) and the Fisher exact test (binary data). The AUC, sensitivity and specificity, and positive predictive values (PPV) and negative predictive values (NPV) were used as performance metrics. Confidence intervals (CI) of the AUC were computed using the bootstrap approach with 1000 iterations. A multivariate logistic regression model was used to combine all features. Only 1 feature from each of the four modalities (EEG, SpO_2, HR, and GA-BW) was included in the regression model, as limiting the number of features eliminates over-training for the model; AUC rankings determined which feature from each modality to include.

Performance of the regression model was assessed using leave-one-infant-out cross validation. This method trains the regression model by fitting parameters from all infants minus one. Performance is then tested on this single left-out infant, and this process is iterated through all infants (363). To eliminate stratification bias caused by unbalanced class proportions in each training iteration, the training set was modified to retrain constant proportions over all iterations. This modification removes, at random and at most, one infant's data per training iteration (364). The cross-validation procedure provides a better estimate of the generalisation performance (the performance on the entire population) compared to the training and testing on the same sample (363).

Odds Ratio (OR) were calculated for each of the four features within the multivariate model and a 95% CI was calculated from the distribution of OR values over all iterations of the cross-validation. A feature significantly contributed to the model if the 95% CI excluded 1. And lastly, the AUC for the multivariate model was compared to the AUC for the clinical course score and EEG grade alone using the bootstrap method in (365). All analyses were performed in MATLAB (version R2013a, The Mathworks Inc., Natick, Massachusetts, United States). All tests were two-sided and a p-value <0.05 was considered statistically significant.

3.3. Results

3.3.1. Subjects

During the study period, 152 preterm infants were born at the CUMH below 32 weeks, of which 81 were enrolled, whilst the others were missed or refused to consent. From the 81 enrolled, 43 preterm infants met the inclusion criteria for this study. Figure 3-3 displaying the breakdown of infants in the study, displaying the number with good or poor EEG, complicated or uncomplicated clinical course and neurodevelopment outcome.

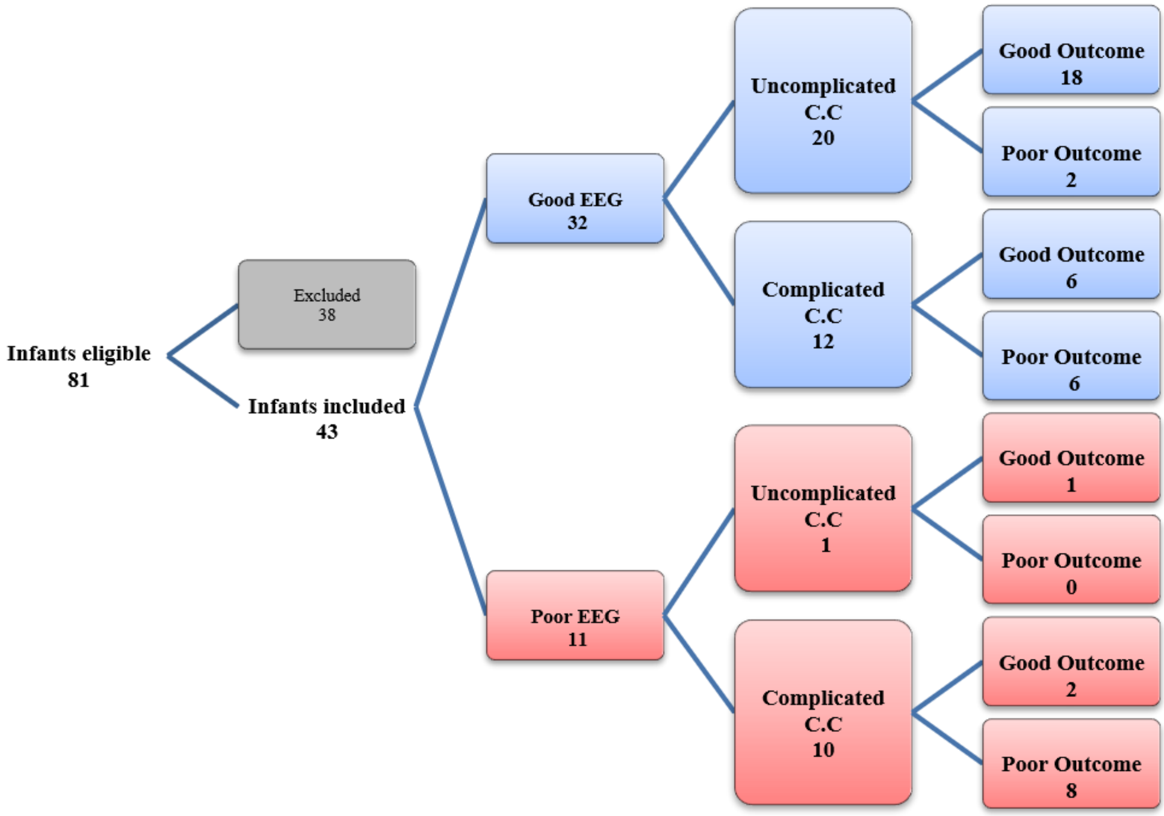


Figure 3-3 Flow chart of the infants who were eligible and included into the study, in addition to their EEG, clinical course and outcome grades/scores.

Recording of simultaneous multimodal physiological data commenced within 24 hours (mean = 8 hours 37 minutes, standard deviation = 5 hours 56 minutes) of birth and continued for up to 72 hours in many cases and longer, if clinically warranted. The mean recording duration was 41 hours 40 minutes (standard deviation = 13 hours 19 minutes). Data at both the 12- and 24-hour time-points was collected from 33 infants, only the 12-hour time-point was collected from three infants and only the 24-hour time-point was collected from 7 infants. Clinical and demographic characteristics and their relationship with outcome are detailed in Table 3-2. GA ranged from 23.42 to 31.86 weeks, with a median (IQR) of 28.71 (26.21 to 29.93) weeks. Morphine or phenobarbitone was given to six infants.

	Good Outcome (n = 27) Median (IQR)	Poor Outcome (n = 16) Median (IQR)	p-value[a]
Gestational age (weeks)	**28.87 (28.29 to 30.14)**	**26.29 (24.86 to 29.57)**	**0.022**
Weight (g)	**1040 (885 to 1327)**	**800 (675 to 1315)**	**0.122**
Apgar score 5 min	**9.0 (8.0 to 9.0)**	**8.0 (5.3 to 8.0)**	**0.001**
Initial pH	**7.2 (7.1 to 7.3)**	**7.2 (7.1 to 7.3)**	**0.88**
	n (%)	n (%)	p-value[b]
Gender			
Male	**7 (26)**	**9 (56)**	**0.059**
Illness			
Grade III/IV IVH or Cystic PVL	**2 (7)**	**5 (31)**	**0.082**
Sepsis	**6 (22)**	**7 (44)**	**0.178**
Necrotizing Enterocolitis	**0 (0)**	**4 (25)**	**0.015**
Chronic Lung Disease	**0 (0)**	**5 (31)**	**0.005**
Retinopathy of Prematurity	**0 (0)**	**0 (0)**	**1**
Mortality	**0 (0)**	**4 (25)**	**0.015**
EEG			
Seizures	**0 (0)**	**3 (19)**	**0.045**
Normal	**24 (89)**	**8 (50)**	**0.010**

TABLE 3-2 CLINICAL DEMOGRAPHICS OF THE INFANTS, AND EEG GRADING COMPARING INFANTS WITH A GOOD AND POOR OUTCOME.

Outcome was defined as neurodevelopmental delay at 2 years of age or death.

[a] Mann-Whitney U-test;

[b] Fischer's exact test

3.3.2. Clinical course score

Twenty-two (51.2%) infants were classified as complicated and 21 (48.8%) infants as uncomplicated based on our clinical grading system.

3.3.3. EEG analysis

Thirty-two infants had a normal EEG (74.4%) and 11 (25.6%) had an abnormal EEG; of these 11, three had seizures. An inter-rate agreement was found for the EEG grading, with a Cohen's kappa coefficient of 0.97.

3.3.4. Outcome Assessment

Four infants died in the neonatal period. Using the Bayley III Scales, 27 (69.2%) surviving infants had a good neurodevelopmental outcome, and 12 (30.8%) had a poor outcome.

Infants with a poor outcome had lower GA (p=0.022) and were more likely to have NEC (p=0.015) or CLD (p=0.005). Table 3-3 provides details of the infants with normal and abnormal EEGs and their normal or abnormal scores for the three Bayley III domain subscales, while figure 3-4 displays a boxplot and median values of the three Bayley III domain subscales.

		Cognitive Scores			
		Normal (n=30)	Abnormal (n=9)	AUC (95% CI)	p-value
EEG1	Normal (n=30)	27	3	0.783 (0.586 – 0.980)	0.011
	Abnormal (n=9)	3	6		

		Language Scores			
		Normal (n=30)	Abnormal (n=7)	AUC (95% CI)	p-value
EEG1	Normal (n=28)	24	4	0.614 (0.368 – 0.861)	0.352
	Abnormal (n=9)	6	3		

		Motor Scores			
		Normal (n=33)	Abnormal (n=4)	AUC (95% CI)	p-value
EEG1	Normal (n=28)	26	2	0.644 (0.334 – 0.954)	0.334
	Abnormal (n=9)	7	2		

TABLE 3-3 CONFUSION MATRIX OF THE EEG NORMALITY AND RELATIONSHIP WITH THE NORMALITY OF THE THREE BAYLEY III DOMAIN SUBSCALES.

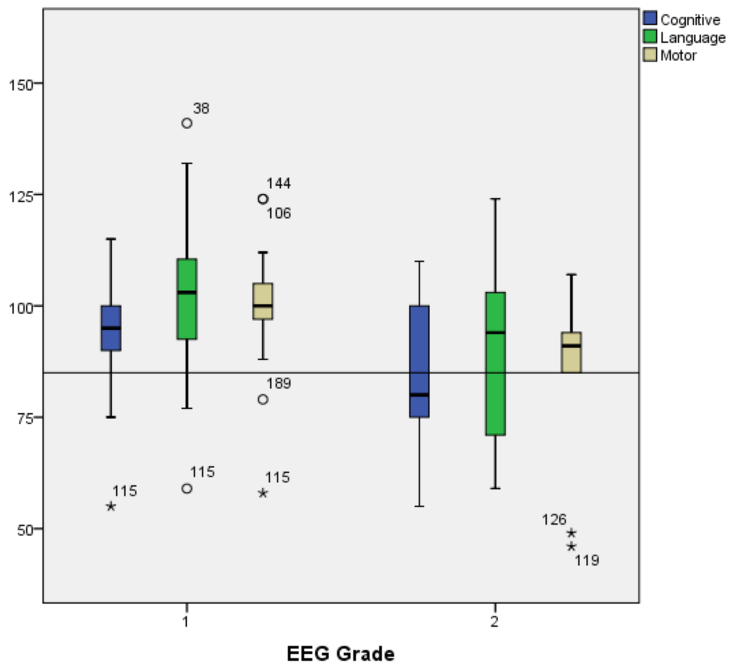


Figure 3-4 Boxplot of scores from all 3 Bayley III domain subscales for infants with normal and abnormal EEG1 recordings. The reference line at 85 illustrates the abnormal test value.

3.3.5. Data analysis

Combining features into a model can improve the performance of our prediction. We selected one feature from each modality group: one from the SpO2 group: one from the HR group: and one features from the age-weight group. A feature ranking table was created to make an informed choice (Table 3-4). The four features selected were mean SpO_2, HR skew, GA and the EEG.

	AUC (95% CI)	p-value[a]	OR (95% CI)
Mean SpO_2*	0.78 (0.62 – 0.90)	0.003	0.71 (0.55 – 0.91)
% time hypoxic	0.68 (0.50 – 0.85)	0.045	1.14 (0.97 – 1.34)
HR: skew*	0.78 (0.63 – 0.92)	0.002	0.54 (0.33 – 0.87)
HR: kurtosis	0.74 (0.55 – 0.89)	0.010	1.05 (0.99 – 1.12)
HR: mean	0.67 (0.50 – 0.82)	0.069	1.06 (1.00 – 1.12)
HR: SD	0.64 (0.44 – 0.81)	0.142	0.90 (0.75 – 1.08)
GA*	0.71 (0.52 – 0.87)	0.022	0.94 (0.90 – 0.99)
Weight	0.64 (0.45 – 0.82)	0.122	1.00 (1.00 – 1.00)
EEG grade*	0.69 (0.55 – 0.83)	0.010	8.00 (1.70 – 37.67)
Clinical course	0.79 (0.66 – 0.90)	<0.001	16.63 (3.05 – 90.67)

TABLE 3-4 FEATURE RANKING TABLE COMPARING FEATURES FOR MODEL INCLUSION.

*Normal outcome n=27, poor outcome n=16. Key: CI, confidence interval; SD, standard deviation; HR, heart rate; AUC, area under the receiver operating characteristic; OR, (odds ratio); *, features included into model.*

[a] Mann-Whitney U-test

These features were combined in the regression model. Their unadjusted and adjusted odds ratios are given in Table 3-5, indicating that all four features are statistically significant in the multivariate logistic regression model.

Features in regression model	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Mean SpO2 (%)	0.71 (0.55–0.91)	0.82 (0.73–0.86)
HR: skew	0.54 (0.33–0.87)	0.63 (0.50–0.73)
GA (days)	0.94 (0.90–0.99)	0.94 (0.92–0.94)
EEG grade	8.0 (1.70–37.67)	2.9 (2.3–4.8)

TABLE 3-5 ODDS RATIO (OR) FOR FOUR FEATURES INDIVIDUALLY (UNADJUSTED OR) AND COMBINED WITHIN THE LOGISTIC REGRESSION MODEL (ADJUSTED OR).

Features are considered statistically significant if the 95% CI excludes 1. The reference EEG grade is the abnormal grade. Key: SpO2, oxygen saturation; CI, confidence interval.

Although some of the features were significantly correlated, the correlation values were small (<0.5) thus making it unlikely that multi-collinearity would affect the regression model. Table 3-6 presents the univariate analysis of these four features as well as the clinical course score, and the regression model.

	AUC (95% CI)	p-value	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Physiological features						
HR: skew	0.78 (0.63–0.92)	0.002[a]	70 (56–87)	69 (54–88)	79 (62–93)	58 (38–82)
Mean SpO2	0.78 (0.62–0.90)	0.003[a]	78 (58–88)	75 (57–90)	84 (67–96)	67 (38–85)
EEG grade	0.69 (0.55–0.83)	0.010[b]	50 (25–73)	89 (75–1)	73 (42–1)	75 (60–91)
Patient demographics						
GA	0.71 (0.55–0.87)	0.022[a]	67 (43–89)	69 (45–88)	78 (55–93)	55 (30–82)
Clinical assessments						
Clinical course Score	0.79 (0.66–0.90)	<0.001[b]	88 (69–1)	70 (53–85)	64 (43–83)	90 (75–1)
Regression model	0.83 (0.69–0.95)		75 (60–93)	74 (60–92)	63 (40–87)	83 (67–96)

TABLE 3-6 UNIVARIATE ANALYSIS AND MULTIVARIATE ANALYSIS FOR PREDICTION OF GOOD AND POOR NEURODEVELOPMENTAL OUTCOME.

Univariate analysis (physiological features, patient demographics and clinical assessments) and multivariate analysis (regression model) for prediction of good (n=27) and poor (n=16) neurodevelopmental outcome (including death). Comparison of the regression model with features of the physiological signals, basic patient demographics and later (clinical course score) clinical assessments. Multivariate analysis for the logistic regression model uses cross-validation. Key: AUC, area under the receiver operator characteristic; PPV, positive predictive value; NPV, negative predictive value; SpO2, oxygen saturation.

[a] Mann-Whitney U-test; [b] Fischer's exact test

Lower GA, lower mean SpO_2, lower HR skew and abnormal EEG grade were predictive of an abnormal outcome. AUC for the regression model is similar to the clinical course score: AUC

(95% CI) for the regression model is 0.83 (0.69-0.95) vs. clinical course score 0.79 (0.66—0.90), p=0.633. Although the regression model has a higher AUC than the AUC of the EEG grade alone 0.69 (0.55—0.83), we find no statistical improvement, p=0.124.

3.4. Discussion

A combination of GA and multimodal physiological signal analysis, recorded within the first 72 hours after birth, has the potential to predict death or neurodevelopmental delay at 2 years of age. The adjusted ORs show that every feature uniquely contributes to the evaluation of outcome and should therefore be included. Although the multimodal model had a larger AUC (0.83) compared to HR skew (0.78), mean SpO_2 (0.78), EEG (0.69) and clinical course score (0.79), the differences failed to reach statistical significance. Lack of statistical significance may be due to the small sample size and hence the low power of the tests. Further studies with larger numbers are required to confirm the results observed in this study. The clinical course score included all relevant clinical information for the entire NICU duration whereas the multimodal model was developed from information obtained in the early transitional period and thus has the advantage of being available in the first few days after birth. This finding highlights the potential value of multimodal monitoring during the transitional period and its possible role in outcome prediction, which could provide useful information for neonatologists in the NICU when guiding early treatment strategies.

Early EEG grade alone demonstrated low sensitivity (50%) and high specificity (89%), highlighting the possible limitation of the EEG grades for the prediction of death or long-term neurodevelopmental delay. These results are consistent with other studies which demonstrated sensitivities of 25 – 61% (293, 315). Although many studies have shown EEG grading to be predictive of long-term outcome, none have shown that simple quantitative features of the readily-available SpO_2 and HR have similar – if not better – performance at predicting 2-year outcome. We find sensitivity and specificity values of 70% and 69% respectively, using quantitative analysis of a HR feature and values of 78% and 75% respectively, using SpO_2 quantitative analysis alone. Abnormal HR variability is associated with fetal and neonatal distress (366). A correlation between abnormal HR variability and clinical signs of sepsis has been reported (360). Sepsis is the main cause of preterm infant death during the first week of life and can also increase vulnerability of the brain due to inflammation and white matter damage (367). Low SpO_2 to the point of hypoxia, can cause tissue damage of the brain which may result in neurological compromise and neurodevelopmental delay (358). The clinical course score had a higher sensitivity (88%) and similar specificity (70%) to the HR feature. The five risk factors included in our clinical course score were chosen a priori as they are associated with long term morbidity. IVH and cPVL are direct injuries to the brain which increase the risk of developing CP and cognitive impairment (368). Developing CLD is another common condition in preterm infants which can also impact on neurodevelopment (369). Neurodevelopment dysfunction is also increased in preterm infants who require surgery for necrotizing enterocolitis (370), who are exposed to sepsis (371), or suffer from severe ROP (372). The clinical course scores were collected at discharge, following diagnosis of any of these major complications, therefore more information, comparative to the early physiological analysis, was available to accurately predict outcome. Yet the multimodal model does provide a more balanced sensitivity—specificity result (75—74%) compared to the clinical score.

Medlock *et al.* found that multivariate models of early clinical information predicted mortality in preterm infants better than BW or GA alone (351). Studies implementing the commonly used SNAP-II and SNAPPE-II scores showed a range of AUC values for the prediction of neonatal mortality, from 0.66 - 0.78 in SNAP-II studies and 0.60 - 0.91 in SNAPPE-II studies (352). These studies concentrated on predicting mortality only, whereas we were also interested in predicting outcome in survivors. Broitman *et al.* found that a model based on clinical variables performed better than a model using head ultrasound for predicting outcome at both 28 days and 36 weeks. Some clinical variables included in this early assessment (by postnatal day 28) were GA and BW (353). Tyson *et al.* demonstrated that a five factor model which consists of GA, BW, gender, exposure to antenatal corticosteroids, and singleton versus twin birth, performed better than GA alone for the prediction of outcome in a cohort of preterm infants between 22 - 25 weeks GA (354). Our AUC results showed an improvement from both these two predictive models (353, 354). Also for our study, the sensitivity, specificity and OR values showed similar values or improvements to previous studies in which EEG or aEEG was evaluated as one predictor or the only predictor (315, 321, 341). However, studies that examined serial EEG recordings or used a larger cohort size had better sensitivity or specificity values (293, 307).

The main limitation of this study is the small sample size and the consequent low statistical power. Although data were collected over a 2-year period in a large maternity hospital, this was a retrospective study and some records had limited EEG data, missing physiological data, or missing outcome data. With low power, only large improvements will reach statistical significance. For example, comparing AUCs between the multivariate model and EEG, we found a difference of 14% but this lacked statistical significance (p=0.12). Another consequence of small numbers is the limit on the number of explanatory variables that the multivariate model can accommodate without over fitting. Because of this limitation, we chose to include only physiological signals in the model in addition to GA, as GA is readily available. Clinical assessments such as Apgar scores were not considered in this study mainly because of this limitation on the number of explanatory variables; but also because of the subjective nature of the score and because this score does not necessarily account for intervention performed in the delivery suite (373). With larger sample sizes, other clinical factors such as respiration, blood pressure, initial pH, lactate, and Apgar, could be explored for inclusion in the multivariate model. Another noticeable limitation is that the multivariate model is not an automated system, as specialist interpretation of the EEG is required. An automated grading system could be developed for preterm EEG similar to available systems for hypoxic-ischaemic encephalopathy in the EEG of term infants (374). In addition, missing data may have had a negative impact on the multimodal model: some infants did not have both 12- and 24-hour data epochs available for analysis, due to either later EEG application or premature discontinuation of monitoring. Although EEG was graded with knowledge of medication history, we did not consider the effects of medication on heart rate and oxygen saturation and thus medication remains a possible confounder in this study. A potential disadvantage of monitoring at such an early stage is that other complications can occur beyond the monitoring period; early monitoring, however, can provide immediate results at the beginning of critical care in the NICU. Serial EEGs and physiological measurements over the infant’s stay in the NICU could add additional predictive information (307).

The main strength of this study is that we are using large amounts of continuous data from different sources. The EEG recordings were reviewed by experienced clinical physiologists that were not involved in the clinical care of the baby and were blinded to the clinical data. This confirmed that the recordings remained anonymous during review. Using EEG instead of the aEEG was a major asset as it provides more valuable second by second data. Although EEG is not readily available in the NICU, all of the other features (HR, SpO_2 and GA) were objective, quantifiable and readily available. This leads to a model which consists of multiple different features. Another strength of this study was that the Bayley Scale of Infant Development III was used to assess all surviving infants, and performed by a physiotherapist, with great experience in performing the assessment.

In conclusion, quantitative analysis of readily available physiological signals, combined with EEG and GA, shows potential for improving our ability to predict death or delayed neurodevelopment at 2 years of age. Early assessment of potential neurological impairment can aid clinical management of the infant. Future studies could consider serial multimodal analysis, including EEG, to monitor maturation and development of EEG features over the first weeks and months of life and their relation to neurodevelopmental outcome.

Chapter 4. Electrographic seizures during the early postnatal period in preterm infants less than 32 weeks

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4.1. Introduction

Seizures are the hallmark of neurological dysfunction but can be difficult to detect and treat in newborns (14). They are an even greater diagnostic challenge in preterm infants where the vast repertoire of general movements can be very difficult to distinguish from the often subtle movements of clinical seizures (324, 375-377). This is compounded further by the high rate of electroclinical dissociation in infants with seizures (378). The early postnatal period, or transitional period, in the preterm infant is of particular concern as the brain is vulnerable to injury and this risk increases with decreasing GA (276). Continuous EEG monitoring is the only way to reliably monitor and treat seizures in newborns but because interpretation is difficult, it is rarely acutely available (379, 380). Many centres instead rely on the aEEG because of its ease of application, maintenance, and interpretation (259). aEEG is a useful tool for assessing neurological function in newborn infants (312) and identifying generalised seizures (259, 381), but it does have limitations. These limitations are greater in the preterm population where the baseline EEG is changing continuously with GA (269), seizures show less generalisation and are of shorter duration (329). Studies based only on clinical diagnosis of seizure frequency in preterm infants range from 3.9 – 57.5 per 1,000 births (320, 382, 383). In very preterm infants, seizure frequency of 0.9 – 8.7% has been reported in EEG studies but have been short duration recordings only or else targeted infants with risk factors for seizures only (307, 324, 325, 384). Much higher seizure frequencies however (22 – 48%) have been reported in the first few days in preterm infants using aEEG (314, 315, 318).

This is the first known study to use continuous, long-duration video-EEG monitoring within the first few days of birth in a population of infants < 32 weeks GA, regardless of their clinical status. Our aim in this chapter therefore was to describe the frequency and characteristics of seizures in preterm infants <32 weeks during the early postnatal period using continuous video-EEG monitoring.

4.2. **Methods**

4.2.1. Participants

For this investigation, infants <32 weeks GA were enrolled from both cohort 1 and 2 (see Figure 2-1). Infants with congenital anomalies were excluded prospectively and infants with EEG recordings <24 hours in duration were excluded retrospectively to optimise the time window for seizure detection. Ethical approval for the collection and analysis of the data was granted by the Clinical Research Ethics Committee of the Cork Teaching Hospitals, Ireland. Written informed parental consent was obtained before recording the EEG.

4.2.2. EEG Recording

The EEG application approach for preterm infants has previously been described in chapter 2. Continuous multichannel video-EEG and a 2 channel aEEG trend (F4-C4 and F3-C3) was recorded as soon as possible after birth when the infant was stable, and continued for up to approximately 72 hours of age, or longer if requested by the clinical team. Three EEG machines were used: the NicoletOne™ EEG system (CareFusion Co., San Diego, USA); the Nihon Kohden, EEG-1200, Neurofax, (Tokyo, Japan); and the Moberg ICU Solutions, CNS-200 EEG and Multimodal Monitor, (Ambler, Pennsylvania). Clinical staff used the aEEG as an aid for clinical assessment. During monitoring, if there were any concerns about suspicious clinical behaviours or aEEG patterns, a neurophysiologists was asked to review the continuous multichannel EEG if possible, but this was dependent on staff availability.

4.2.3. Seizure analysis

The entire video-EEG recording for each infant was reviewed and all seizures were identified and annotated independently by an electroencephalographer (RL[8]). Another electroencephalographer (EP[9]) also annotated all EEGs with seizures. A third electroencephalographer (GB[10]) reviewed a subset of all recordings and reviewed any seizures where disagreement existed to provide a consensus. A seizure was defined as a

[8] Rhodri Lloyd
[9] Elena Pavlidis
[10] Geraldine Boylan

clear ictal event comprising a sudden, repetitive, evolving stereotyped waveform, that had a definite start, middle and end and lasting for at least 10 seconds (385). Figure 4-1 demonstrates how a seizure starts and evolves into a clear rhythmical high amplitude seizure. The onset and offset of each electrographic seizure was annotated and exported to text files for further analysis.

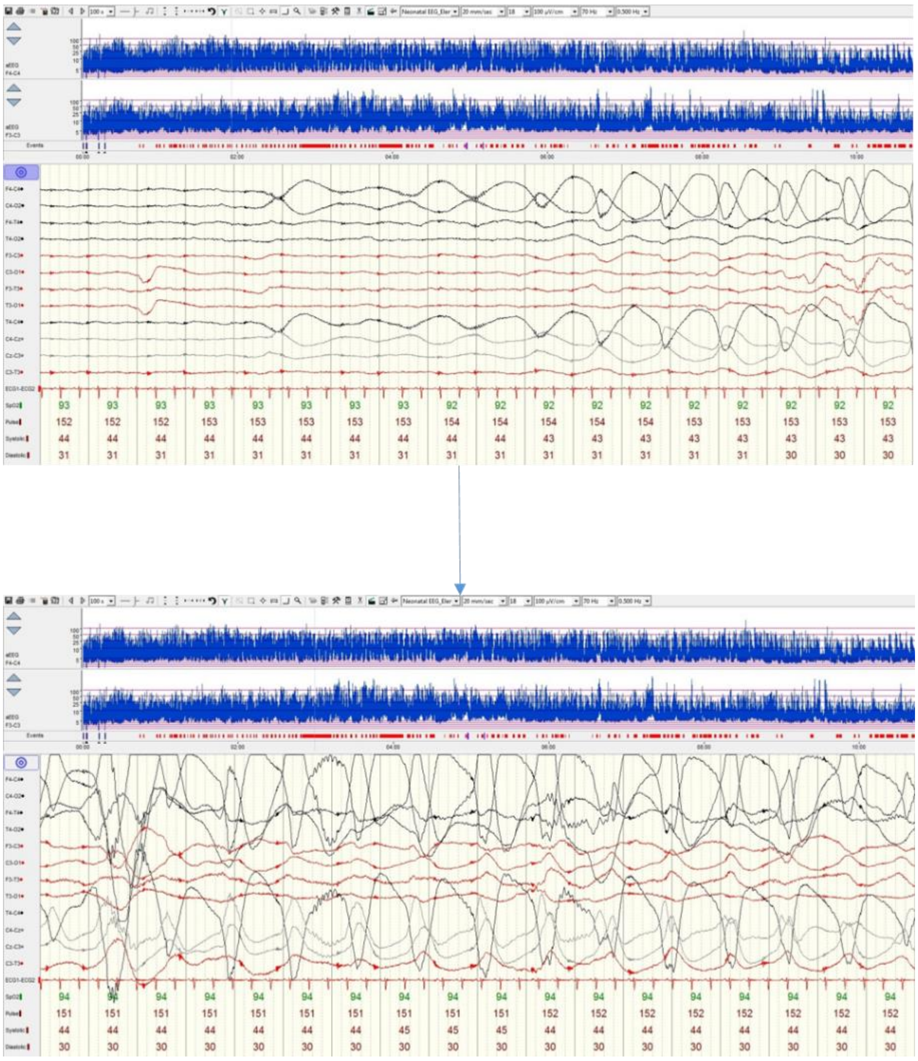

Figure 4-1 Example of a seizure in a preterm infant, illustrating how a seizure starts gradually and becomes more pronounced with high amplitude rhythmical activity.

4.2.4. Seizure Characteristics

Several seizure characteristics were calculated from the annotation text files. These metrics are illustrated in (Figure 4-2) and described as follows. Total seizure number is the total number of seizures over the entire recording. Mean seizure duration is the mean duration of all seizures in the EEG record in seconds. Total seizure burden is the total duration of all seizures in the entire recording. Seizure onset is the start time of the first recorded seizure. Total seizure period is the time between seizure onset and the end of the last recorded seizure. Maximum seizure burden and time of maximum seizure burden are the maximum point, and time (postnatal age) of the maximum point, of the temporal distribution of seizure burden. This distribution, also known as instantaneous seizure burden, is calculated as the midpoint of a 1-hour window (seizure burden per hour) shifted in time, by 1 second, across the entire EEG record (386, 387). Seizure burden per hour is the total seizure duration, in minutes, within a 1-hour window.

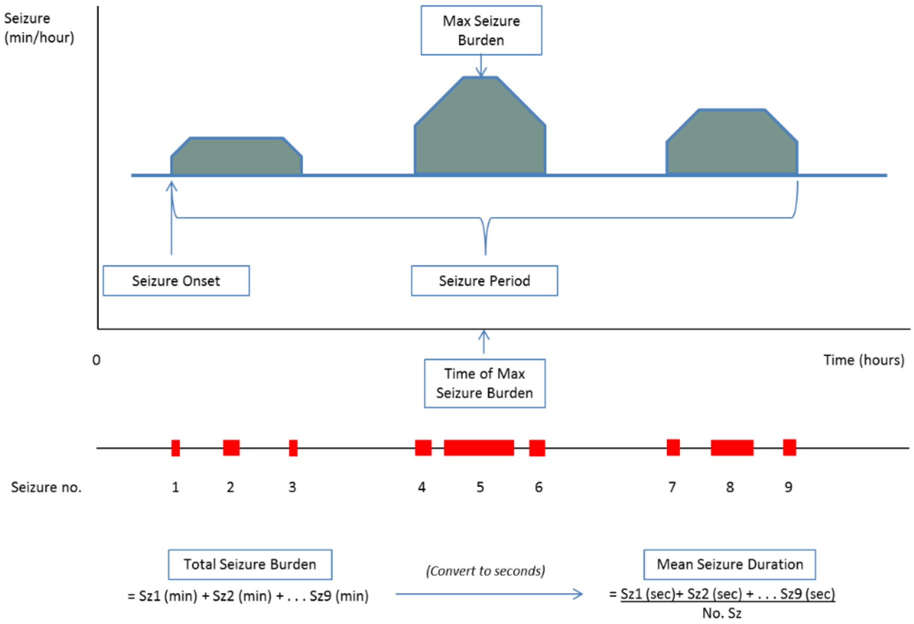

Figure 4-2 Metrics to characterise the temporal evolution of seizures for each infant. Metrics include: total seizure burden, mean seizure duration, time of seizure onset, total seizure period, maximum seizure burden, and time of maximum seizure burden.

For each seizure, the onset location, morphology and evolution was described (388). Video-EEG analysis provided information on clinical seizure manifestations and were categorized as either electrographic or electroclinical. Electroclinical seizures were described as clonic, tonic, myoclonic, spasms, autonomic or subtle (boxing, pedaling, oral automatisms, ocular movements), following Volpe's modified classification (14, 389). Annotations also allowed the identification of any periods of status epilepticus, defined as continuous or accumulative electrographic seizures present in more than 50% of a 1 hour period (390).

4.2.5. Additional data collection

Serial CRUS scans were collected for IVH grading or presence of cPVL. Grade 3 or 4 IVH, cPVL were considered to be significant brain abnormalities. As per our standard clinical practice, all scans were officially performed and reported by a Paediatric Radiologist who was not involved in the study and was blinded to EEG data. Infants had the first CRUS within the first 72 hours of birth where possible, with repeat scans between 7 – 10 days of age and at one month of age. Timings would vary slightly depending on the availability of the Clinical Paediatric Radiologist and the infants' clinical condition. Each infants GA, BW, Apgar at 1 and 5 minutes and mechanical ventilation (intubation in the delivery suite and mechanical ventilation over first 3 days of age) were collected. Additionally, we calculated the CRIB II, which is a clinical risk instrument with scores ranging from 0–27 (42). It provides an index of risk based on sex, GA, BW, admission temperature, and base deficit in the first blood sample. AEDs were administered at the clinicians' discretion. Phenobarbitone was the first-line drug of choice, administered intravenously as a loading dose of 20 mg/kg. The timing of administration was recorded, along with the administrations of other drugs such as caffeine and analgesics.

4.2.6. Statistical Analysis

Continuous data were described using median and interquartile ranges (IQR). Differences between the non-seizure and seizure groups were tested using the Mann-Whitney *U* test and Fisher's exact test. All analyses were performed in SPSS Statistics 21 (SPSS Inc, Chicago, Illinois). All tests were two-sided and a p-value <0.05 was considered to be statistically significant.

4.3. Results

4.3.1. Subjects

A total of 288 infants were admitted to the NICU during the two recruitment periods, and 120 infants were enrolled (Figure 4-3). From cohort 1, 53 infants (29%) were included from a possible 185, while from cohort 2, 67 (65%) were included from a possible 103.

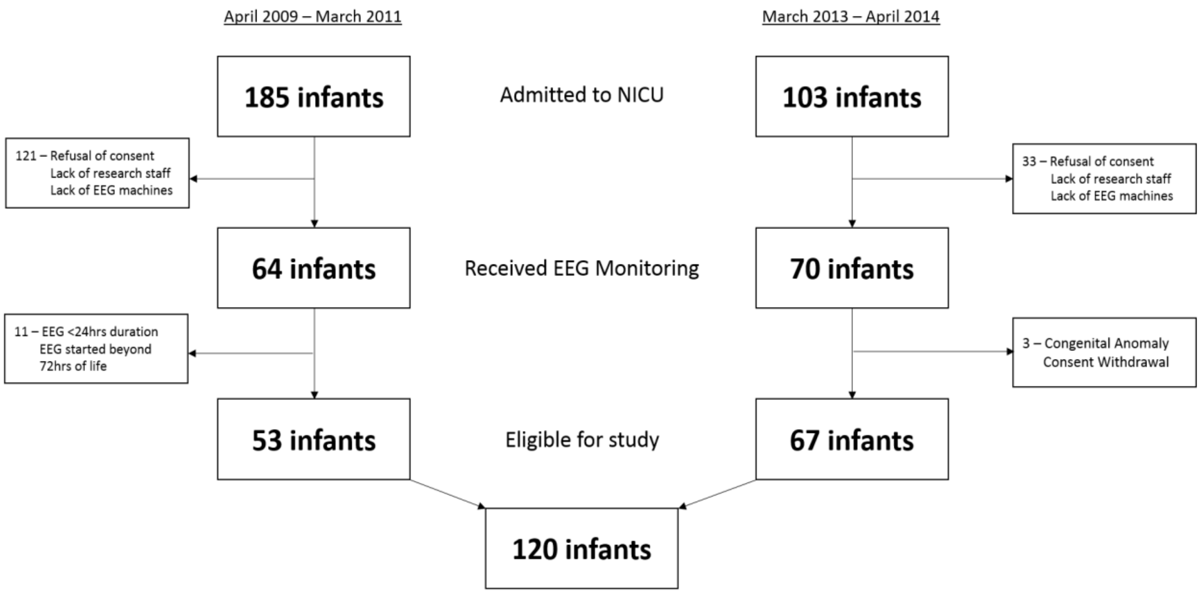

Figure 4-3 Flow chart of the study population. Indicates the number of infants admitted to the NICU, commenced EEG monitoring and enrolled for the study, from the two recruitment periods.

The higher percentage of babies enrolled in the second recruitment period was due to the availability of more research staff and EEG machines for monitoring. Nine infants (7%) were excluded retrospectively as the EEG recordings were <24 hours in duration. EEG commenced at a median postnatal age of 7 hours (IQR: 4 hours 37 minutes – 10 hours 47 minutes) with 46 infants (38%) commencing within 6 hours of age. The median recording duration was 59 hours 49 minutes (IQR: 46 hours 57 minutes – 69 hours 9 minutes). It was possible to record the EEG for up to 72 hours of age in 51% of infants only, due to reasons including, delayed EEG application and early EEG removal at the clinician or parents' request. In total, 6,932 hours of EEG was visually analysed.

4.3.2. Seizure Analysis

Six infants developed electrographic seizures in the early postnatal period, with a total of 307 seizures; see examples in Figures 4-4A, 4-4B and 4-4D. Figures 4-4B and 4-4D show electrographic seizures (from infant 1 and 6, respectively) which were evident in both the multichannel EEG and aEEG, whilst Figure 4-4A shows an electrographic seizure (from infant 1) only evident on the multichannel EEG. Figure 4-4C, the only non-seizure figure, displays muscle artefact on the EEG and an associated raised aEEG baseline. In this example, multiple raised baselines are evident on the aEEG, a feature often associated with seizures, however

EEG confirmed that this infant had only one seizure (Figure 4-4D); other periods of raised aEEG baseline were a mixture of biological artefact and state change. Of the 307 seizures, 97 (32%) were clearly evident on the aEEG. Other seizures were not clear due to brief duration or localised region of onset.

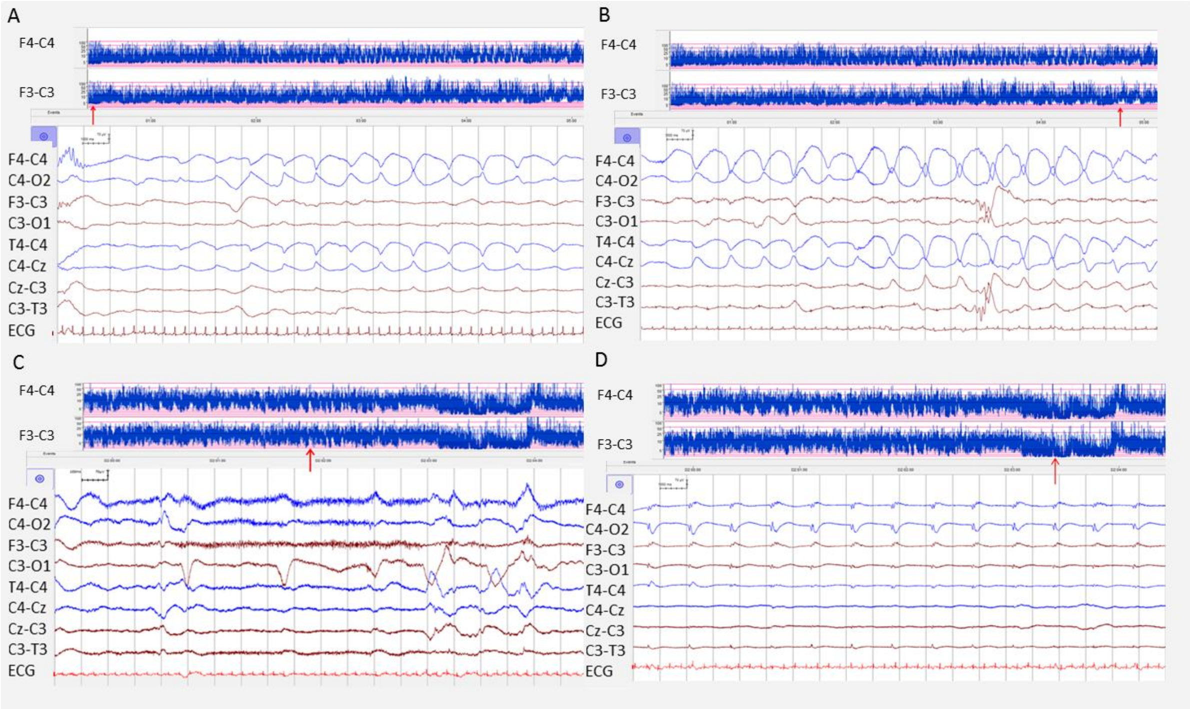

Figure 4-4 Multichannel EEG/aEEG recording, displaying seizure identification challenges with aEEG. All four figures display multichannel EEG-aEEG on day one (arrows indicate corresponding aEEG). Infant 1. A: Seizure, with a high amplitude rhythmical delta, is displayed on the EEG, but not on aEEG. B: Seizure displayed on both EEG (high amplitude rhythmical delta) and aEEG. Infant 6. C: Artefact on EEG corresponds to the raised aEEG baseline. D: Seizure, of a low amplitude spike and slow wave, is displayed on the EEG with the corresponding aEEG only displaying a slightly raised baseline.

Table 4-1 compares the clinical demographics of infants with and without seizures. Three of the 6 infants with seizures had evidence of significant CRUS abnormality (infant 1, 4 and 6): in two infants, grade IV IVH occurred during the first 72 hours; the other infant developed a grade I IVH during the monitoring period which progressed to a bilateral grade III IVH by day 10. Significant CRUS abnormalities occurred in 11 other infants who did not have seizures in the first 72 hours. In five, IVH grades III/IV were identified in the first 72 hours. Three infants who had normal CRUS during EEG monitoring, had cPVL abnormalities at days 24, 29 and 31. One of these infants developed sepsis when seven days old. In 2 other infants, CRUS was only performed after the end of the EEG monitoring period at which point, CRUS abnormalities were already evident, whilst another infant had a normal CRUS at 24 hours which was abnormal when repeated at day 5.

	Infants without Seizures (n=114)	**Infants with Seizures (n=6)**	**p-value**
GA (weeks) Median (IQR)	28.9 (26.6 – 30.3)	25.7 (24.3 – 29.1)	0.043 [a]
BW (g) Median (IQR)	1135 (848 – 1443)	885 (570 – 1308)	0.142 [a]
Gender = Male Total (%)	58 (51)	3 (50)	0.644 [b]
Apgar 1 min Median (IQR)	7 (5 – 8)	2 (0.75 – 2.75)	<0.001 [a]
Apgar 5 min Median (IQR)	8 (7 – 9)	5 (4.25 – 7)	<0.001 [a]
CRIB II score Median (IQR)	7 (4 – 10)	11.5 (7.5 – 4)	0.032 [a]
Intubation in delivery suite Total (%)	35 (31)	6 (100)	0.001 [b]
First day mechanical ventilation Total (%)	73 (64)	6 (100)	0.076 [b]
Mechanical ventilation from day 1 through to day 3 Total (%)	42 (37)	6 (100)	0.003 [b]
IVH I/II Total (%)	28 (25)	2 (33)	0.469 [b]
IVH I/II in first 72 hrs Total (%)	10 (9)	1 (17)	0.446 [b]
IVH I/II after 72 hrs Total (%)	6 (5)	0 (0)	0.730 [b]
IVH I/II timing unknown Total (%)	12 (11)	1 (17)	0.505 [b]
Significant Brain Abnormality Total (%)	11 (10)	3 (50)	0.021 [b]
IVH III/IV in first 72 hrs Total (%)	5 (4)	2 (33)	0.039 [b]
IVH III/IV after 72 hrs Total (%)	0 (0)	1 (17)	0.050 [b]
IVH III/IV timing unknown Total (%)	3 (3)	0 (0)	0.856 [b]
cPVL Total (%)	3 (3)	0 (0)	0.856 [b]
Chronic lung disease Total (%)	24 (20)	3 (50)	0.127 [b]
Sepsis Total (%)	52 (46)	4 (67)	0.279 [b]
Necrotizing enterocolitis Total (%)	22 (19)	1 (17)	0.676 [b]
Retinopathy of prematurity Total (%)	2 (2)	0 (0)	0.902 [b]
Death Total (%)	6 (5)	2 (33)	0.051 [b]

TABLE 4-1 CLINICAL DEMOGRAPHICS OF THE INFANTS, COMPARING INFANTS WITH AND WITHOUT SEIZURES. Key: GA, gestational age; BW, birth weight; g, grams; min, minutes; IQR, interquartile range; IVH, intraventricular haemorrhage; cPVL, cystic periventricular leukomalacia.

[a] – Mann Whitney U-test, [b] – Fischer's exact test

4.3.3. Seizure Characteristics

More detailed demographic and clinical information about the six infants with seizures, as well as the characteristics of these seizures are shown in Table 4-2.

	Infant						
	1	2	3	4	5	6	Total
Gestational age (weeks)	30	24	23	25	28	26	–
Weight (g)	1450	540	580	850	1260	920	–
Apgar at 5 minute	5	2	6	7	5	7	–
Sex	M	F	F	M	M	F	–
Intraventricular haemorrhage	D2: I D10: III	–	D13: II	D2: IV	D3: II	D1 :IV	5
Death	–	Y	–	Y	–	–	2
AED	PB	–	–	PB	–	–	2
Morphine	–	–	–	Y	Y	Y	3
Caffeine	Y	Y	Y	–	–	Y	4
Number of seizures (number)	**151**	**6**	**85**	**49**	**15**	**1**	**307**
Location of seizure onset							
Frontal	80	–	25	2	4	–	111 (36%)
Central	8	–	18	2	4	1	33 (11%)
Temporal	39	–	5	2	–	–	46 (15%)
Occipital	24	6	37	42	7	-	116 (38%)
Seizure Morphology							
Rhythmical Delta (High Amplitude)	74	–	–	–	–	–	74 (24%)
Rhythmical Delta (Low Amplitude)	73	–	11	–	11	–	95 (31%)
Rhythmical Sharp Delta/Theta	–	6	–	–	–	–	6 (2%)
Rhythmical Alpha (Low Amplitude)	4	–	–	–	–	–	4 (1%)
Sharp Waves (Periodic)	–	–	–	49	–	–	49 (16%)
Sharp & slow wave (Low Amplitude)	–	–	–	–	4	–	4 (1%)
Spike & slow wave (Low Amplitude)	–	–	–	–	–	1	1 (<1%)
Spike & slow wave (High Amplitude)	–	–	74	–	–	–	74 (24%)
Seizure Type							
Video Unobtainable	**–**	**5**	**–**	**3**	**8**	**–**	**16 (5%)**
Classifiable seizures	**151**	**1**	**85**	**46**	**7**	**1**	**291 (95%)**
Clonic	8	–	63	12	5	–	88 (30%)
Myoclonic	31	–	21	7	–	1	60 (21%)
Subtle Boxing and/or Pedalling	–	–	–	2	–	–	2 (<1%)
Tonic	45	–	–	4	–	–	49 (17%)
Only Electrographic	67	1	1	21	2	–	92 (32%)

TABLE 4-2 PRETERM INFANTS WITH SEIZURES: DEMOGRAPHIC, CLINICAL AND ELECTROCLINICAL CHARACTERISTICS. Key: M, male; F, female; D, day; Y, yes; N, no; PB, Phenobarbitone 20mg/kg.

Seizure semiology could not be categorised for 5% of all electroclinical seizures as the video was obscured by blankets, humidity of the incubator, or handling. Ninety-two of the 291 seizures recorded using video (32%) were electrographic only and 68% were electroclinical. Electrographic seizure onset in all infants was focal: frontal in 36%; central in 11%; temporal in 15%; and occipital in 38%. Seventy-nine percent of seizures spread contralaterally, the remainder showed some ipsilateral cortical spread, therefore no seizures were isolated to one channel only. Seizure type varied between infants. Generally, rhythmic delta/theta activity constituted 58%, whilst 42% were sharp/spike and slow waves. The most frequent electroclinical seizure types were myoclonic/clonic (51%) and tonic (17%). One infant had two periods of status epilepticus as per the definition commonly used (390). Temporal seizure characteristics for each infant are described in Table 4-3 and Figure 4-5.

	Infant						
Seizure Temporal Characteristic	1	2	3	4	5	6	**Median (IQR)**
Age at start of record (hh:mm)	06:29	07:10	10:01	13:00	28:55	07:46	
Number of seizures	151	6	85	49	15	1	32.0 (8.3 to 76.0)
Total Seizure burden (mins)	213.8	2.4	67.6	134.1	12.9	0.7	40.3 (5.0 to 117.5)
Mean seizure duration (s)	84.9	24.2	47.7	164.1	51.5	42.0	49.6 (43.4 to 76.6)
Seizure period (h)	11.0	17.1	15.0	29.7	8.4	<0.1	13.0 (9.1 to 16.6)
Seizure onset (h)	7.5	20.1	14.6	31.9	40.2	21.3	20.7 (16.0 to 29.3)
Max Seizure burden (min/h)	36.3	0.5	21.1	17.3	4.3	0.7	10.8 (1.6 to 20.2)
Time of max seizure burden (h)	9.7	20.7	24.0	54.2	40.8	21.3	22.7 (20.9 to 36.6)

TABLE 4-3 TEMPORAL CHARACTERISTICS OF SEIZURES FOR EACH INFANT.

Two of the 6 infants with seizures received AEDs during EEG monitoring. These infants had the highest seizure burden, and the majority of their seizures were electroclinical. One infant received a loading dose of PB prior to monitoring, due to high clinical suspicion of seizures. He had a severe perinatal asphyxia, with a normal CRUS and the EEG showed an isoelectric background but no seizures. Additionally, caffeine was administered to 60% of infants during the first 72 hours, including 4 of the infants who had seizures.

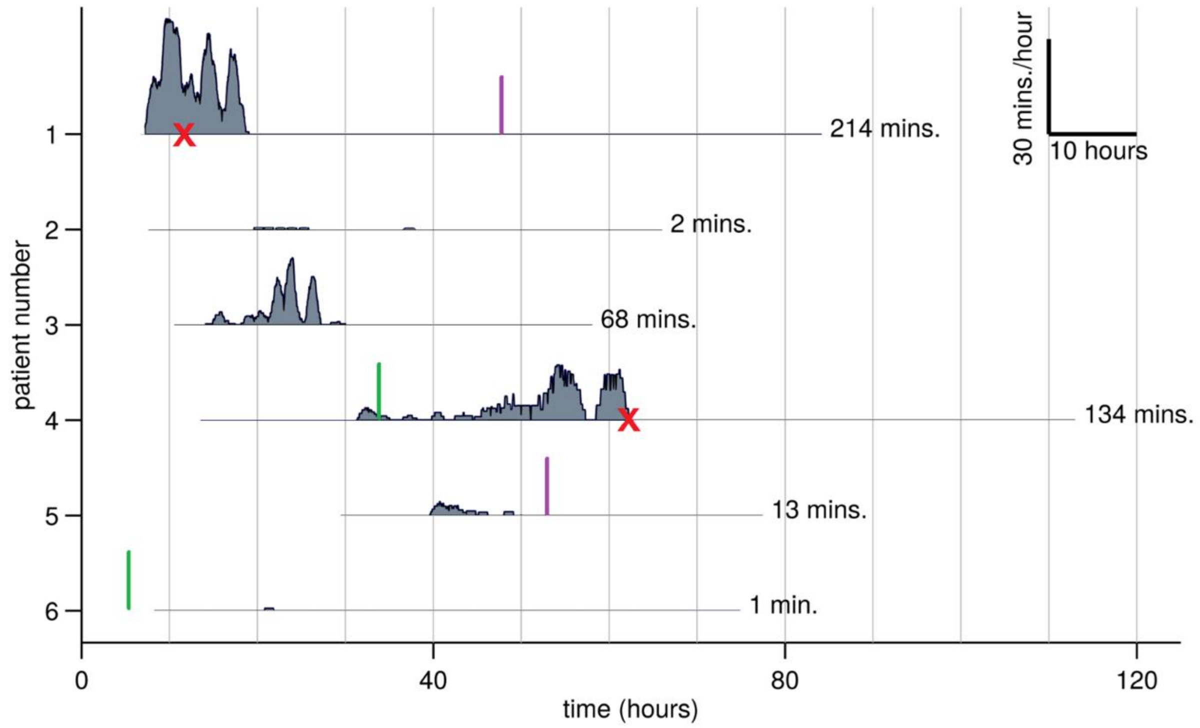

Figure 4-5 Distribution of instantaneous seizure burden over time for the 6 infants with electrographic seizures. Time is post-birth and grey horizontal lines represent recording time for the EEG. Red crosses represent the approximate timing of first administration of phenobarbitone, the purple lines indicate the approximate time when IVH grade 1 or 2 was identified on CRUS and the green line indicates the approximate time IVH grade 3 or 4 was identified on CRUS. Total seizure burden for each infant is displayed at the end of each horizontal line.

4.4. Discussion

In this chapter, we report the first study to use continuous, long duration, video-EEG monitoring to qualitatively and quantitatively describe electrographic seizures in preterm infants <32 weeks during the early postnatal period. Seizures were observed in only 5% of our population, a much lower frequency than that reported using aEEG studies of very preterm infants in the early postnatal period (314, 315, 318).

It is difficult to directly compare our results with previous EEG studies in preterm infants as few have used continuous long term EEG monitoring in the first few days after birth (310, 391). In our study, multichannel video-EEG monitoring commenced as soon as possible after birth and continued for a median recording duration of approximately 60 hours. Four multichannel EEG studies of infants <32 weeks GA reported similar low seizure rates (0.9, 3, 3.9, and 8.7% respectively) but used only short-duration EEG recordings of approximately 1 hour (307, 324, 325, 384). Two of these studies recorded EEGs at varying times after birth, specifically at the time when risk factors or clinical signs of seizures were suspected (325, 384). In the remaining two studies, EEGs were recorded either during the first week (range 1 – 43 days) (324) or during the first three days after birth (307).

Seizure identification can be challenging in preterm infants as background EEG patterns vary considerably with GA and rhythmic patterns are common. Seizure duration is shorter in preterm compared to term infants, which makes seizure recognition using aEEG alone very difficult (321). We discovered a median seizure duration of 49.6 seconds. Furthermore, similar to other reports, all seizures in our study had a focal onset (209, 324), predominantly over frontal and occipital regions (209, 388). Frontal and central cortical regions are often used for aEEG monitoring, and seizures originating in other regions can be missed. In our investigation, seizures originating and/or spreading to temporal and occipital regions were also seen. Additionally, seizures did not generalise and only spread to adjacent brain regions, which would make recognition on aEEG especially challenging. Biological and external artefacts, high amplitude rhythmical slow activity, different GA and state changes may also lead to misinterpretation of the aEEG (269-271, 392, 393). Artefacts such as movement, muscle, respiration and hiccup can all raise the aEEG baseline, mimicking seizures, as seen in Figure 4-4C (175) so it is particularly important to interrogate the raw EEG traces (as available on most aEEG machines) to identify seizures (394). Sixty-eight percent of seizures identified on EEG were not clearly identifiable on the aEEG, however further work on aEEG detection of preterm seizures is clearly warranted in a larger study that includes more infants with seizures. Multichannel video-EEG, in our opinion, is the most useful tool for preterm EEG monitoring as it provides more comprehensive spatial information, ensuring that seizures originating over all cortical regions can be identified. Video can help identify unusual movements and artefacts and most EEG systems have the ability to synchronously record other physiological variables such as heart rate, oxygenation, respiration and blood pressure which provide much needed additional information for preterm EEG interpretation.

The most common electroclinical seizure types seen were clonic (30%) and myoclonic (21%). In the two infants with the highest seizure burden, both had more than one type of electroclinical seizure, similar to a previous study by Pisani et al (395). Thirty-two percent (92/291) of all seizures in six infants were electrographic only and 53% (49/92) of these occurred following AED administration, highlighting the need for continuous EEG monitoring in preterm infants in order to measure the efficacy of treatment. Four infants with seizures were not treated, possibly because of low seizure burden, short individual seizure duration, no clinical manifestations or vital parameter changes during the seizures. PB was administered to one infant with severe perinatal asphyxia in the non-seizure group due to a high clinical suspicion of seizures in the first hour after birth, however no seizures were seen in the subsequent EEG monitoring period. We do not know if seizures were abolished with PB or if the movements seen were because the infant was in a very abnormal neurological state. This infant had a severely abnormal background EEG pattern and subsequently died. Early EEG monitoring from as soon as possible after birth is very useful in preterm neonates with perinatal asphyxia but understandably this is not always possible, particular if the infant is unstable.

Seizure durations of 128 to 546s, total seizure burdens of 49 to 224 min and seizure periods of 16.5 – 36.6 h have been reported in studies of full term and older preterm infants (321, 384-387, 390, 396, 397). In our small group of preterm infants, seizure duration, total seizure burden and seizure period were much shorter. Janáčková et al. also reported similar seizure durations (median of 52s) in 56 preterm infants born between 24 – 36 weeks GA (with recordings between 27 - 39 weeks corrected age) when compared to 46 full-term infants (329).

All infants in our investigation were assigned a CRIB II score which has been shown to predict outcome in very preterm infants (43). We found higher CRIB II scores in those infants who subsequently developed seizures which may be useful when deciding which preterm infants to monitoring with EEG. Seizures appeared more prominent in younger infants (median age of 25.7 weeks). This might be related to the fact that different GAs can influence seizure frequency and onset, as previously suggested by Sheth et al (398). Another finding was that intubation immediately in the delivery suite was associated with seizures, which was also similarly reported in a study by Pisani et al. (325, 384). Additionally, seizures showed an association with both Apgar at 1 and 5 minutes, as previously reported by Davis et al, who stated that infants with seizures were likely to have a 5-minute Apgar of ≤ 4 (326). A high seizure frequency (45 – 65%) has previously been reported in preterm infants with high grade IVH (315, 318, 395). Our results show that 7 out of 120 infants developed severe IVH (grade III and IV) in the first few days and only 2 of these had seizures on the EEG during this period. However, the seizure burden was highest in these 2 infants suggesting some association between seizure burden and IVH in preterm neonates. Five infants without seizures also developed high grade IVH during EEG monitoring. As CRUS imaging was not performed every day in our infants, it was difficult to estimate the exact timing of IVH. In both infants who had IVH grade III/IV and seizures, CRUS images were performed early, and both infants developed post haemorrhagic ventricular dilatation by day 13. Electrographic seizures were also seen in a 24-week GA infant with normal CRUS, although the seizure burden was much lower at 2.4 minutes. We recommend the use of early multichannel video-EEG where possible for preterm infants of lower gestation, with low Apgar scores, higher CRIB II scores, and evidence of brain injury on CRUS, as our data suggest that these are the infants at higher risk of seizures (see Table 4-1). Previous studies have also reported seizures in association with brain injury (314, 315, 318, 395), lower GA and BW (318) and other clinical conditions such as sepsis, metabolic disorders and perinatal asphyxia (395).

During the early postnatal period when the infant is adapting to extrauterine life, cerebral autoregulation may be poor and cerebral blood flow can fluctuate, increasing vulnerability to neurological injury (54, 399, 400) and risk of later disability (276). The goal of neonatal intensive care is to support the infant and protect the developing brain. Electrical brain activity in preterm infants occurs in characteristic bursting patterns which are essential for neuronal survival and development (121). Seizures disrupt these patterns, and if sustained, may interrupt neuronal survival and connectivity and contribute to longer term neurodisability (121-123). EEG monitoring is therefore essential to reliably diagnose and treat seizures as soon as possible, to restore baseline electrical patterns and to avoid treating infants unnecessarily. Indeed, concern does exist about the potential neurotoxic effects of phenobarbitone and AED therapy (124-126). A study by Bittigau et al. reported an association between cognitive impairment and reduced brain mass following AED exposure in early life and cautioned the over use of AEDs in preterm infants (127). In our cohort, only one infant was treated purely on clinical suspicion of seizures, before multichannel EEG commenced. This low rate is likely due to the availability of early aEEG/EEG. In our experience, the use of multichannel EEG in the NICU setting helps prevent unnecessary AED treatment in preterm infants in particular as it provides detailed information about the rapidly evolving electrographic activity of the preterm brain and helps identify seizures more accurately particularly in situations where clinical assessment or aEEG recordings are inconclusive.

The major strength of this investigation is the prospective recruitment of 120 infants <32 weeks in the early postnatal period over a three-year period in a level 3 neonatal intensive care unit. We used long term, multichannel video-EEG monitoring to continuously monitor cerebral function and detect seizures. This is the gold standard for detection, quantification and characterization of neonatal seizures and for assessing AED response. Nevertheless, some limitations were evident. We were unable to monitor every infant during the recruitment period, as refusal to participate and/or lack of EEG machines or staff limited those who were recruited. The percentage of infants enrolled was also lower in the first recruitment period as only one researcher and one EEG machine was available on a 24 hour basis. However, selection was not based on the infants' clinical status and all eligible infants were approached if staff were available. Therefore, although we cannot definitely exclude a selection bias, we do not think our results would be very different as we did not select infants based on their clinical status. We aimed to record the EEG for up to 72 hours of age in all infants and this was achieved in 51%. Occasionally the EEG was removed at the clinician or parents' request for a variety of reasons. In some cases, during early stabilisation, it was not always possible or appropriate to apply the EEG electrodes soon after delivery and EEG recording onset was later. However, at least 24 hours of EEG recording within the first 72-hour window was achieved in all infants included in our analysis, and 75% had at least 48 hours of recording. We cannot of course exclude the possibility that some seizures might have been missed in infants who had <48 hours of EEG monitoring within the first 72 hours of age. EEG monitoring during the first 6 hours can be particularly challenging but we did achieve this in 46 infants (38%) and no seizures were seen in this period. Therefore, although we cannot completely exclude some briefer or minor seizures during the period when we did not have EEG ongoing, we do not think that a significant number of seizures were missed. Within our cohort, electrographic seizures were observed in only 6 infants, therefore our quantitative and qualitative descriptors of seizures are based on this small number. Repeat studies on the basis of clinical condition and CRUS change, would be useful, as seizures in preterm infants can also occur later as reported previously (267, 307, 395, 401). Nevertheless, recording in the early postnatal period is important as the brain is particularly susceptible to neurological injury and seizures may be the only manifestation of altered neurological function.

This chapter has highlighted that the frequency of seizures during the early postnatal period, as quantified with continuous multichannel video-EEG monitoring, was low in this cohort of infants compared to previous studies. Whilst all infants in the seizure group were intubated in the delivery suite, our opinion is that infants of lower gestation, with low Apgar scores, higher CRIB II scores and evidence of brain injury on CRUS are at higher risk of seizures, suggesting that these infants might be considered for early EEG monitoring. In this population, if the aEEG is concerning, we recommend the use of multichannel video-EEG, as per the guidelines of the American Clinical Neurophysiology Society (171). This will help avoid both over and under diagnosis and unnecessary exposure to potentially neurotoxic AEDs during a very vulnerable period of brain development.

Chapter 5. A standardised assessment scheme for conventional EEG in preterm infants.

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5.1. Introduction

Neonatal EEG scoring systems have previously been developed with the intention of providing clinical information to the NICU staff (194, 402). Although the EEG characteristics of preterm and term infants are vastly different, the existing EEG assessment systems have been developed for mixed populations of both preterm and term infants. These assessment schemes lack the identification of specific preterm EEG features, which develop with PMA. This is evident in a recently developed system, named the 'standardized computer-based organised reporting of EEG' (SCORE), which provides a standard way of reporting EEG without attempting to grade the EEG for prognostic purposes (403). As this is a system targeting infants at all age groups, there are no specific EEG features defined for preterm infants at varying post-menstrual age (PMA). A specific EEG scoring system for very preterm infants was recently developed to predict neurodevelopmental outcome, however this is a multimodal evaluation which included the EEG surveillance, clinical assessment at discharge and cerebral imaging for outcome assessment (309). Other studies similarly attempted to predict outcome, in which the previous EEG grading system by Holmes (402), was used on populations of preterm infants, although not designed specifically for preterm infants (321, 395). Conventional EEG is the gold standard for assessing brain activity and has been extensively proven to be related to outcome in both term and preterm infants (194, 288, 289, 307, 308, 404-408). However, these studies lacked a systematic approach to the developing features of the preterm EEG for prognostic purposes.

Due to the increasing survival rates of very and extremely preterm infants, there is an urgent need to provide well-defined boundaries between normal and abnormal EEG features at different PMA and to objectively evaluate brain activity and maturation.

Therefore, the aim of this chapter is to develop a method, which is as objective as possible, to evaluate and analyse normal and abnormal EEG features in preterm infants, at different ages and to assess the interobserver agreement of this method when tested by two experts independently.

5.2. Materials and methods

5.2.1. Development of EEG assessment scheme

5.2.1.1. Preliminary Literature Review

To develop an EEG analysis scheme specifically for preterm infants, a comprehensive literature review was performed to identify existing descriptions and definitions of both normal and abnormal EEG features in preterm infants. This involved identifying descriptions and definitions of specific normal and abnormal waveforms/features recognisable in preterm EEG recordings. PUBMed was used to complete searches and filters were applied to eliminate any 'non-human' studies. No language restrictions were applied. We included studies from the year 1990 onwards. Authors (RL and EP [11]) initially searched for the literature independently. In addition, secondary sources of data (such as references used in papers) and personal libraries were also included. This was undertaken in order to ensure that all previously published preterm EEG features described in relevant papers were included in our objective assessment scheme (174, 175, 194, 277, 292, 309, 402, 409, 410).

5.2.1.2. Original EEG assessment scheme design

Following the literature review, an EEG scheme was developed with accompanying instructions for use. The instructions provided definitions for all the EEG features at specific PMA (six different age groups, according to the existing literature (175)) and guidance on how to grade any specific abnormal waves and features into mild, moderate and severe. The EEG features were divided into 4 categories: (temporal organisation/cyclicity, normal waves, abnormal waves, and abnormal features). Definitions of the waveforms/features were adjusted dependant on the age group they appeared. Certain waveforms/features do not appear in all ages, therefore were not considered in certain age groups. The EEG scheme was developed to simplify and guide preterm EEG evaluation at the cot side. The scheme involves scoring 1 or 0 to note the presence or absence of the required features and grading into mild, moderate and severe for specific features.

[11] Rhodri Lloyd and Elena Pavlidis

5.2.1.3. Neurophysiological data – EEG procedure

The patients from Cohort 1 (specifically mentioned in Chapter 2) were retrospectively recruited for this study. The EEG application procedure was the same for cohort 1 as it was for cohort 2 and is explained in chapter 2. The only difference being that silver-silver chloride electrodes were used and that the only machine used was the NicoletOne EEG system (CareFusion Co., San Diego, USA). EEG application was performed after consultation with the medical and nursing staff and when the infant was clinically stable, before continuous video-EEG monitoring commenced.

5.2.1.4. Patients

Preterm infants less than 37 weeks GA who underwent continuous, conventional EEG monitoring in the first 3 days of life between April 2009 and March 2011 were retrospectively selected. The only exclusion criterion was the presence of major congenital malformations. Ethical approval was obtained by the Clinical Research Ethics Committee of the Cork Teaching Hospitals, Ireland. Written informed parental consent was obtained.

In total, three steps of analysis and testing was undertaken to develop the assessment scheme to a satisfactory standard.

5.2.2. First - step analysis (Data Group 1)

For analysis purposes, and to ensure a widely distributed range of EEG data, we selected four different time-points at 12, 24, 48, 72 hours of age. Two-hour epochs of EEG were pruned at all four time-points and subsequently used for analysis. For the first-step analysis, the developed assessment scheme was assessed independently by two electroencephalographer observers skilled in neonatal EEG evaluation (RL and EP[12]). The two observers were blinded for all information except PMA, time of EEG recording post-delivery and whether there was administration of morphine, phenobarbitone or other AEDs during the EEG monitoring. Kappa score for inter-rater agreement was calculated for each infant at each time point. When differences occurred between the two observers, the

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EEG/features were reviewed for any disagreements. Following this, the assessment scheme was reviewed and edited in order to better explain/clarify the parameter.

5.2.3. Second - step analysis (Data Group 2)

In preparation for the second step analysis, further infants were randomly selected at random time-points between 12, 24, 48, 72 hours for two-hour EEG analysis. The revised, second version of the assessment scheme, along with the EEGs, were assigned to two expert professors with vast experience in neonatal and premature EEG analysis (GB and FP[13]). These experts were not involved in the previous stages of development or analysis. The two experts used the EEG assessment scheme to interpret the EEG, while being blinded to all information except for GA, administration of morphine, phenobarbitone or other AEDs during EEG and time of EEG recording post-delivery. Examination was performed independently, and any difficulties in implementing the assessment scheme were identified. Disagreements were discussed and suggestions were made for further modifications to the assessment scheme. Following this, the final version of the assessment scheme was confirmed.

5.2.4. Third - step analysis (Data Group 3)

Further infants were randomly selected for the third-step analysis. The final version of the assessment scheme were used to analyse the selected EEG recordings. The same two experts independently graded the two-hour recordings of each infant at random time-points. Again, the two experts were blinded to all information except for PMA, administration of morphine, phenobarbitone or other AEDs or during EEG and time of EEG recording post-delivery. Normal and mild abnormal features were grouped together for statistical purposes, as previously performed in other studies K-scores for interrater agreement were calculated.

[13] Geraldine Boylan and Francesco Pisani

5.2.5. Statistical Analysis

Continuous data were described using the median and IQR and categorical data with number and percentage. In the first and third step analysis, kappa values were calculated per-infant, while in the third step analysis, percentage difference were calculated per-infant, and median percentage agreement (IQR) values were calculated for both <30 week and >30 week group of infants. Furthermore, an independent samples t-test was used to compare the kappa means for both age groups.

Percentage agreement was defined as the ratio of the number of agreements to the sum of agreements plus disagreements within a category. For each feature, agreement was defined as both experts assigning the same score to an infant, while disagreement was defined as the two experts assigning a different score to an infant. Each individual features were also investigated, with a kappa score and percentage difference calculated per feature. The kappa score was not computed for some features that lacked variability within the experts' examination. For each infant, percentage agreement between the two experts was calculated for each EEG category (temporal organisation/cyclicity, normal waves, abnormal waves and abnormal features).

Furthermore, median percentage agreement was compared between the normal and abnormal features using a Wilcoxon signed-rank test. For this analysis, the mildly abnormal features were grouped with the normal features, as previously performed in chapter 3.

The statistical analysis was performed using SPSS Statistics 21 (SPSS Inc, Chicago, Illinois). The statistical test was 2-tailed and a p-value < 0.05 was considered to be statistically significant.

5.3. Results

5.3.1. Preliminary step

An EEG assessment scheme was developed with maturation specific features for four different groups of PMA (23-25, 26-27, 28-29, 30-31 weeks). The preliminary step led to the development of the first draft of the age-specific assessment scheme, seen in Figure 5-1. It comprised four categories of EEG features, namely: 1)) temporal organisation, 2) normal features, 3) abnormal waves and 4) abnormal features. The normative values and definitions of each EEG feature can vary depending on the PMA group.

	23-25wks	26-27wks	28-29wks	30-31wks
Group 1 (Temporal Organisation)	**Cyclicity**	**Cyclicity**	**Cyclicity**	**Cyclicity**
	IBI	**IBI**	**IBI**	**IBI**
	Burst	**Burst**	**Burst**	**Burst**
Group 2 (Normal Features)	**STOPS**	**STOPS**	**PTT**	**PTT**
	Delta	**PTT**	**Delta Brushes**	**Delta Brushes**
	Theta	**Delta**	**Delta**	**Delta**
	Sharp theta	**Theta**	**Theta**	**Theta**
Group 3 (Abnormal Waves)	**PRS**	**PRS**	**PRS**	**PRS**
	PTS	**PTS**	**PTS**	**PTS**
	Frontal/Occipital Sharp	**Frontal/Occipital Sharp**	**Frontal/Occipital Sharp**	**Frontal/Occipital Sharp**
	Central/Temporal Sharps	**Central/Temporal Sharps**	**Central/Temporal Sharps**	**Central/Temporal Sharps**
	Deformed waves	**Deformed waves**	**Deformed waves**	**Deformed waves**
	Immature Waves	**Immature Waves**	**Immature Waves**	**Immature Waves**
Group 4 (Abnormal Features)	**Asymmetry**	**Asymmetry**	**Asymmetry**	**Asymmetry**
	Asynchrony	**Asynchrony**	**Asynchrony**	**Asynchrony**
	Isoelectric	**Isoelectric**	**Isoelectric**	**Isoelectric**
	Burst Sup	**Burst Sup**	**Burst Sup**	**Burst Sup**
	BIRDs	**BIRDs**	**BIRDs**	**BIRDs**
	Seizures	**Seizures**	**Seizures**	**Seizures**

Figure 5-1 First version of the assessment scheme with allocated spacing for scoring the EEG based on the features.

Instructions were also generated in order to give normative values, detailed definitions of the features and modalities in order to give scores appropriately for the different features in each specific group of age. The instructions contain guidance for grading specific abnormal waves (such as immature waves and deformed waves and mechanical brushes, grading their amount in: few, moderate or several) and for abnormal features (such as low voltage and discontinuity, graded in: mild, moderate or severe). The normative values and definitions of each EEG feature can vary depending on the PMA group.

5.3.2. First - step analysis

The same observers used the first edition of the assessment scheme to grade 8 infants (data group 1) (range PMA: 24+0 – 31+4 weeks) at 12-, 24-, 48- and 72-hour time-points, where available. Of the 8 infants, two infants were observed at each PMA grading groups 23-25, 26-27, 28-29, 30-31 weeks). The clinical characteristics of these infants are evident in table 5-1.

	Infants First Step Analysis (n=8) Median (IQR)
GA (weeks)	27.1 (25.8 – 29.3)
BW (g)	830 (677.5 – 977.5)
Apgar score 5 min	8 (7.5– 9)
CRIB II	9 (6.8 – 10.8)
Initial pH	7.00 (7.10 – 7.27)
	n (%)
Gender (Male)	4 (50)
IVH Grade III/IV /cPVL	2 (25)
Sepsis	4 (50)
NEC	1 (13)
CLD	1 (13)
ROP	0 (0)
AEDs	1 (13)
Morphine	2 (25)
Mortality	1 (13)

TABLE 5-1 CLINICAL DEMOGRAPHICS OF THE INFANTS INCLUDED IN THE FIRST STEP ANALYSIS. Key: IQR, interquartile range; GA, gestational age; BW, birth weight; g, grams; min, minutes; IVH, intraventricular haemorrhage; cPVL, cystic periventricular leukomalacia; NEC, necrotizing enterocolitis; CLD, chronic lung disease ROP, retinopathy of prematurity; AEDs, anti-epileptic drugs.

Almost perfect agreement (0.81 – 1) was achieved in 5 out of the 8 neonates, while substantial agreement (0.61—0.8) was obtained in two infants and moderate agreement (0.41 – 0.6) was obtained in one infant (Table 5-2).

Patients	Kappa

1	0.84
2	0.58
3	0.93
4	0.90
5	0.75
6	0.82
7	0.89
8	0.65
K values according to Landis: < 0: no agreement 0–0.20: slight 0.21–0.40: fair 0.41–0.60: moderate 0.61–0.80: substantial 0.81–1: almost perfect agreement.	

TABLE 5-2 K-SCORES BETWEEN THE TWO OBSERVERS FROM THE FIRST STEP ANALYSIS.

Following the observers practical experience of the new assessment scheme, further evaluation was undertaken. Explanations on PRS, PTS, asymmetry and asynchrony were improved, a section for the documentation of any medication that could affect the EEG was included, and a section for observer annotations were included, to document and highlight anything of interest.

5.3.3. Second - step analysis

The experts reviewed 2-hour epoch recordings from data group 2, which consisted of 24 infants (range GA: 23+3 – 31+4 weeks) at 12, 24, 48, or 72 hours after birth. The 24 infants consisted of six infants from 4 different PMA groups: (23-25, 26-27, 28-29, 30-31 weeks). These were randomly selected for the second-step analysis of the EEG data. One EEG time-point (12, 24, 48 or 72 hours) were randomly selected for observation.

The clinical characteristics of these infants are evident in table 5-3.

	Infants Second Step Analysis (n=24) Median (IQR)
GA (weeks)	27.8 (28.0 – 29.7)
BW (g)	890 (673 – 1105)
Apgar score 5 min	8 (6 – 10)
CRIB II	9 (7 – 13)
Initial pH	7.23 (7.15 – 7.28)
	n (%)
Gender (Male)	10 (42)
IVH Grade III/IV /cPVL	6 (25)
Sepsis	5 (21)
NEC	3 (13)
CLD	6 (25)
ROP	0 (0)
AEDs	2 (4)
Morphine	5 (21)
Mortality	1 (4)

TABLE 5-3 CLINICAL DEMOGRAPHICS OF THE INFANTS INCLUDED IN THE SECOND STEP ANALYSIS. Key: IQR, interquartile range; GA, gestational age; BW, birth weight; g, grams; min, minutes; IVH, intraventricular haemorrhage; cPVL, cystic periventricular leukomalacia; NEC, necrotizing enterocolitis; CLD, chronic lung disease ROP, retinopathy of prematurity; AEDs, anti-epileptic drugs.

Agreements for this analysis were not calculated as the two experts discussed the issues in order to improve the standardised features to be included in the EEG assessment scheme. Following this step, some features were modified and some new features were added in order to improve preterm EEG characterization. Continuity was a feature added to the temporal organisation group and was available in the 26 – 27 week and older but not the 23 – 25 PMA group, due to lack of persistent continuous activity periods in this age group. This was included to identify normal duration and amplitude of continuous activity at different PMA. A feature added to all PMA groups was the level of abnormal discontinuity. In the instructions, a description for mild, moderate and severe discontinuity were included, and in the scheme the ability to indicate the correct grade is available. The same was applied to grade abnormal voltage, where mild, moderate and severe were again the options. Two new features that were added for all PMA groups were Status Epilepticus and Periodic Lateralized Epileptiform Discharges (PLEDS). Burst Suppression was removed in the younger PMA groups and only included from 30 weeks PMA and older. This decision was made as currently there is uncertainty in how to define Burst Suppression in the younger PMA, as previously suggested by some authors (403). Additionally, immature waves were removed from the 23 – 25 weeks PMA group, due to lack of information about a normal EEG <23 weeks PMA. Certain features were combined together to simplify the scheme. Theta and sharp theta were combined in the normal features, while all the sharp waves were grouped together. STOPS and Occipital Sawtooth waves were grouped together, as they are similar features with similar clinical relevance. The deformed waves feature was adapted also, to ungroup deformed waves and mechanical brushes to be two independent features in the scheme. Finally, it was decided to add two new age groups: 32 -34 and 35 – 36 weeks, to cover the entire range of premature infants. All changes led to the amendments of the assessment scheme. The final version of the assessment scheme, following the internal evaluation of the two electroencephalographers and the evaluation of the two experts, is evident in Figure 5-2. The following pages (p.171 -176) includes the full instructions of the final version of the assessment scheme.

	23-25wks	26-27wks	28-29wks	30-31wks	32-34wks	35-36wks
Group 1 (Temporal Organisation)	**Cyclicity**	**Cyclicity**	**Cyclicity**	**Cyclicity**	**Cyclicity**	**Cyclicity**
	IBI	**IBI**	**IBI**	**IBI**	**IBI**	**IBI**
	Burst	**Burst**	**Burst**	**Burst**	**Burst**	**Burst**
		Continuity	**Continuity**	**Continuity**	**Continuity**	**Continuity**
Group 2 (Normal Waves)	**STOPS/ Occipital**	**STOPS/ Occipital**				**Delta**
	sawtooth	**sawtooth**	**PTT**	**PTT**	**PTT**	**Brushes**
	Delta	**PTT**	**Delta Brushes**	**Delta Brushes**	**Delta Brushes**	**Delta**
	Theta	**Delta**	**Delta**	**Delta**	**Delta**	**Theta**
		Theta	**Theta**	**Theta**	**Theta**	**Frontal transient**
					Frontal transient	**SAD**
Group 3 (Abnormal Waves)	**PRS**	**PRS**	**PRS**	**PRS**	**PRS**	**PRS**
	PTS	**PTS**	**PTS**	**PTS**	**PTS**	**PTS**
	Sharps	**Sharps**	**Sharps**	**Sharps**	**Sharps**	**Sharps**
	Deformed waves	**Deformed waves**	**Deformed waves**	**Deformed waves**	**Deformed waves**	**Deformed waves**
	Mechanical brushes	**Mechanical brushes**	**Mechanical brushes**	**Mechanical brushes**	**Mechanical brushes**	**Mechanical brushes**
		Immature Waves	**Immature Waves**	**Immature Waves**	**Immature Waves**	**Immature Waves**
Group 4 (Abnormal Features)	**Asymmetry**	**Asymmetry**	**Asymmetry**	**Asymmetry**	**Asymmetry**	**Asymmetry**
	Asynchrony	**Asynchrony**	**Asynchrony**	**Asynchrony**	**Asynchrony**	**Asynchrony**
	Discontinuity	**Discontinuity**	**Discontinuity**	**Discontinuity**	**Discontinuity**	**Discontinuity**
	Low Voltage	**Low Voltage**	**Low Voltage**	**Low Voltage**	**Low Voltage**	**Low Voltage**
	Isoelectric	**Isoelectric**	**Isoelectric**	**Isoelectric**	**Isoelectric**	**Isoelectric**
				Burst Sup	**Burst Sup**	**Burst Sup**
	BIRDs	**BIRDs**	**BIRDs**	**BIRDs**	**BIRDs**	**BIRDs**
	PLEDs	**PLEDs**	**PLEDs**	**PLEDs**	**PLEDs**	**PLEDs**
	Seizures	**Seizures**	**Seizures**	**Seizures**	**Seizures**	**Seizures**
	Status	**Status**	**Status**	**Status**	**Status**	**Status**
Medications						
Annotations						

Figure 5-2 The final version of the assessment scheme with allocated spacing for scoring the EEG based on the features

EEG ASSESSMENT SCHEME for PRETERM INFANTS

Colours division

Temporal organization/Cyclicity
Normal waves
Abnormal waves
Abnormal features

Abbreviations:

AS = Active sleep
F trans = Frontal transients
IBI = Inter-bursts interval
PRS = Positive Rolandic Sharps
PTS = Positive Temporal Sharps
QS = Quiet sleep
SAD = Slow anterior dysrhythmia
STOP pattern = Sharp theta on the occipitals of prematures

***(For immature waves, deformed waves and mechanical brushes, it is possible to insert a comment on their amount (few, moderate, several))**

23 – 25 weeks

Cyclicity: Not observed.
IBI: < 60 sec, < 15 µV.
Burst: Delta-theta activity, < 60 sec, > 50 µV (often > 300 µV).
STOP pattern: sharp theta on the occipitals of prematures / Occipital Sawtooth: rhythmic, regularly shaped, medium-high amplitude 4±7 Hz activities, lasting 0.5±3 sec and located in the occipital regions.
Slow Delta: - > 300 µV & 0.3 - 1 Hz; - Mono-/Diphasic, smooth (with superimposed fast activity on C-O regions); - Central and Occipital: uni- or bi-lateral, Temporal: uni- (with right predominance) or bilateral sequences; Frontal: less represented.
Theta: Diffuse or temporal bilateral bursts of theta activity (often sharp).
Positive Rolandic Sharps (PRS): > 0.1 per min.
Positive Temporal Sharps (PTS): 400 ms & > 50 µv & > 0.1 per min.
Sharps (Frontal, Central, Temporal and/or Occipital): > 100 µV & > 0.1 per min.
Mechanical brushes: Spindle-like, fast spiky wave bursts with maximal amplitudes higher than 40 µV and frequencies between 13 and 20 Hz.
Deformed delta: Lack of smoothness, wider basis, increased peak-to-peak amplitude.
Asymmetry: > 50% difference in amplitude and/or frequencies between the 2 hemispheres and/or if pathological waves are exceeding in one hemisphere for > 50% compared to the other.
Asynchrony: Normally frequent. Sign as pathological only if constantly present for high amplitude bursts.
Mild discontinuity: Slightly prolonged IBI for at least 50% of a 1 hour-period.
Moderate discontinuity: Moderate increase of discontinuity OR increase discontinuity with maximal IBI less than one and a half the maximal value for the age group for at least 50% of a 1 hour-period.
Severe discontinuity: Very long IBI, non-reactive OR maximal IBI above one and a half the maximal value for the age group for at least 50% of a 1 hour-period.
Mildly Low voltage: Delta waves < 200 µV (at least 50% of a 1 hour-period).
Moderately Low Voltage: Rare waves between 50 - 100 µV, most < 50 µV (at least 50% of a 1 hour-period).
Severely abnormal Low Voltage: persistent ≤ 10 µV (at least 50% of a 1 hour-period).
Isoelectric tracing: Mainly inactive tracing with activity < 5 µV.
Brief Intermittent Rhythmic Discharges (BIRDs): Paroxysms of a seizure-like rhythmic electrographic activity with a duration < 10 sec.
Periodic lateralized epileptiform discharges (PLEDs): Sharp waves or spikes that repeat periodically with an almost regular interval, lateralized to one hemisphere and showing no clear evolution in terms of frequency/morphology and amplitude.
Seizures: Sudden, repetitive, evolving and stereotyped ictal pattern with a clear beginning, middle, and ending and a minimum duration of 10 sec.
Status epilepticus: 30 min of seizure activity OR recurrent seizures with no recovery for at least 30 min OR 50% seizure burden in 1 hour period.

26 – 27 weeks

Cyclicity: Not observed.
IBI: < 60 sec, < 30 µV.
Burst: Delta-theta activity, >50uV (often > 300 µV).
Brief periods of semi-continuous activity: Up to 80 sec; mainly runs of high amplitude (> 300 µV) delta activity with very low frequency (0.3 - 1 Hz) on the occipital regions.
STOP pattern: Sharp theta on the occipitals of prematures / Occipital Sawtooth: rhythmic, regularly shaped, medium-high amplitude 4±7 Hz activities, lasting 0.5±3 sec and located in the occipital regions.
Premature temporal theta (PTT) or "temporal sawtooth'': Runs of rhythmic theta activity of 4.5 - 6 Hz (starts to appear).
Slow Delta: - > 300 µV & 0.3 - 1 Hz; - Diphasic Delta - Central and occipital. In the occipital regions: high amplitude, smooth or with sparse theta/alpha superimposed.
Theta: - Approximately 200 µV - Diffuse / predominant in temporal regions - Can be sharp.
Positive Rolandic Sharps (PRS): > 0.1 per min.
Positive Temporal Sharps (PTS): 400 ms & > 50 µv & > 0.1 per min.
Sharps (Frontal, Central, Temporal and/or Occipital): > 100 µV & > 0.1 per min.
Mechanical brushes: Spindle-like, fast spiky wave bursts with maximal amplitudes higher than 40 µV and frequencies between 13 and 20 Hz.
Deformed delta: Lack of smoothness, wider basis, increased peak-to-peak amplitude.
Immature waves: Presence of waves which are usually seen in previous ages.
Asymmetry: > 50% difference in amplitude and/or frequencies between the 2 hemispheres and/or if pathological waves are exceeding in one hemisphere for > 50% compared to the other.
Asynchrony: Normally frequent. Sign as pathological only if constantly present for high amplitude bursts.
Mild discontinuity: Slightly prolonged IBI OR mildly decreased semi-continuous activity for at least 50% of a 1 hour-period.
Moderate discontinuity: Moderate increase of discontinuity OR increase discontinuity with maximal IBI less than one and a half the maximal value for the age group for at least 50% of a 1 hour-period.
Severe discontinuity: Very long IBI, non-reactive OR maximal IBI above one and a half the maximal value for the age group for at least 50% of a 1 hour-period.
Mildly Low voltage: Delta waves < 200 µV (at least 50% of a 1 hour-period)
Moderately Low Voltage: Rare waves between 50 - 100 µV, most < 50 µV (at least 50% of a 1 hour-period).
Severely abnormal Low Voltage: persistent ≤ 10 µV (at least 50% of a 1 hour-period).
Isoelectric tracing: Mainly inactive tracing with activity < 5 µV.
Brief Intermittent Rhythmic Discharges (BIRDs): Paroxysms of a seizure-like rhythmic electrographic activity with a duration < 10 sec.
Periodic lateralized epileptiform discharges (PLEDs): Sharp waves or spikes that repeat periodically with an almost regular interval, lateralized to one hemisphere and showing no clear evolution in terms of frequency/morphology and amplitude.
Seizures: Sudden, repetitive, evolving and stereotyped ictal pattern with a clear beginning, middle, and ending and a minimum duration of 10 sec.
Status epilepticus: 30 min of seizure activity OR recurrent seizures with no recovery for at least 30 min OR 50% seizure burden in 1 hour period.

28 – 29 weeks

Cyclicity: AS - QS outlined.
IBI: ≤ 30 sec (40 sec might be accepted if occasional), < 30 µV.
Burst: Longer 0.3 - 1Hz, sometimes > 300 µV, can have superimposed brushes.
Continuous activity: Up to 160 sec; 20 - 300 µV delta and theta activity.
Premature temporal theta (PTT) or "temporal sawtooth'': Runs of rhythmic theta activity of 4.5 - 6 Hz.
Delta Brushes: Random or briefly rhythmic delta waves (0.3 - 1.5 Hz), with an amplitude of 50 - 300 µV, and superimposed bursts of fast rhythms (> 8 Hz) of 10 - 60 µV. They start to appear (few).
Slow Delta: -30 - 300 µV & 0.5 - 2 Hz; - Diphasic Delta - Less diffuse (rarely anterior), abundant in central > 1 sec; posterior predominance, synchrony in occipital - up to 20 sec.
Theta: - 20 - 260 µV - Synchronised diffuse bursts or temporo-occipital - Sharp when temporal - Frequent.
Positive Rolandic Sharps (PRS): > 0.1 per min.
Positive Temporal Sharps (PTS): 400 ms & > 50 µv & > 0.1 per min.
Sharps (Frontal, Central, Temporal and/or Occipital): > 100 µV & > 0.1 per min.
Mechanical brushes: Spindle-like, fast spiky wave bursts with maximal amplitudes higher than 40uV and frequencies between 13 and 20 Hz.
Deformed delta: Lack of smoothness, wider basis, increased peak-to-peak amplitude.
Immature waves: Presence of waves which are usually seen in previous ages.
Asymmetry: > 50% difference in amplitude and/or frequencies between the 2 hemispheres and/or if pathological waves are exceeding in one hemisphere for > 50% compared to the other.
Asynchrony: Normally frequent. Sign as pathological only if constantly present for high amplitude bursts.
Mild discontinuity: Slightly prolonged IBI OR mildly decreased continuous activity for at least 50% of a 1 hour-period.
Moderate discontinuity: Moderate increase of discontinuity OR increase discontinuity with maximal IBI less than one and a half the maximal value for the age group for at least 50% of a 1 hour-period.
Severe discontinuity: Very long IBI, non-reactive OR maximal IBI above one and a half the maximal value for the age group for at least 50% of a 1 hour-period.
Mildly Low voltage: Delta waves < 200 µV (at least 50% of a 1 hour-period).
Moderately Low Voltage: Rare waves between 50 -100 µV, most < 50 µV (at least 50% of a 1 hour-period).
Severely abnormal Low Voltage: persistent ≤ 10 µV (at least 50% of a 1 hour-period).
Isoelectric: Mainly inactive tracing with activity < 5 µV.
Brief Intermittent Rhythmic Discharges (BIRDs): Paroxysms of a seizure-like rhythmic electrographic activity with a duration < 10 sec.
Periodic lateralized epileptiform discharges (PLEDs): Sharp waves or spikes that repeat periodically with an almost regular interval, lateralized to one hemisphere and showing no clear evolution in terms of frequency/morphology and amplitude.
Seizures: Sudden, repetitive, evolving and stereotyped ictal pattern with a clear beginning, middle, and ending and a minimum duration of 10 sec.
Status epilepticus: 30 min of seizure activity OR recurrent seizures with no recovery for at least 30 min OR 50% seizure burden in 1 hour period.

30 – 31 weeks

Cyclicity: AS (continuous/semi-continuous activity) - QS (discontinuous)
IBI: ≤ 20 sec (in QS)
Burst: ≥ 3 sec, 0.5 - 1.5 Hz, 100-200 µV, can have superimposed brushes.
Continuous activity.
Premature temporal theta (PTT) or "temporal sawtooth'': runs of rhythmic theta activity of 4.5 - 6 Hz (more during QS).
Delta Brushes: Delta waves (0.5 - 1.5 Hz), with an amplitude of 50 - 300 µV, and superimposed bursts of fast rhythms (> 8 Hz) of 10 - 60 µV rhythms. Diffuse.
Diphasic Delta: - 100 - 200 µV (sometimes up to 300 µV) & 0.5 - 2 Hz - Always superimposed by faster rhythms - Occipital or occipito-temporal, mainly synchronous, more during AS.
Theta: - > 25 µV & 4.5 - 6 Hz - Mainly temporal and in QS.
Positive Rolandic Sharps (PRS): > 0.1 per min.
Positive Temporal Sharps (PTS): 400 ms & > 50 µv & > 0.1 per min.
Sharps (Frontal, Central, Temporal and/or Occipital): > 100 µV & > 0.1 per min.
Mechanical brushes: Spindle-like, fast spiky wave bursts with maximal amplitudes higher than 40 µV and frequencies between 13 and 20 Hz.
Deformed delta: Lack of smoothness, wider basis, increased peak-to-peak amplitude.
Immature waves: Presence of waves which are usually seen in previous ages.
Asymmetry: > 50% difference in amplitude and/or frequencies between the 2 hemispheres and/or if pathological waves are exceeding in one hemisphere for > 50% compared to the other.
Asynchrony: Still normal. Delta waves are mainly synchronous.
Mild discontinuity: Slightly prolonged IBI OR mildly decreased continuous activity for at least 50% of a 1 hour-period.
Moderate discontinuity: Moderate increase of discontinuity OR increase discontinuity with maximal IBI less than one and a half the maximal value for the age group for at least 50% of a 1 hour-period.
Severe discontinuity: Very long IBI, non-reactive OR maximal IBI above one and a half the maximal value for the age group for at least 50% of a 1 hour-period.
Mildly Low voltage: Delta waves < 200 µV (at least 50% of a 1 hour-period).
Moderately Low Voltage: Rare waves between 50 - 100 µV, most < 50 µV (at least 50% of a 1 hour-period).
Severely abnormal Low Voltage: persistent ≤ 10 µV (at least 50% of a 1 hour-period).
Isoelectric: Mainly inactive tracing with activity < 5 µV.
Burst suppression: Bursts of theta and/or delta waves (sometimes with fast activity) alternating with periods of low amplitude activity (< 20 uV). No reactivity to stimuli.
Brief Intermittent Rhythmic Discharges (BIRDs): Paroxysms of a seizure-like rhythmic electrographic activity with a duration < 10 sec.
Periodic lateralized epileptiform discharges (PLEDs): Sharp waves or spikes that repeat periodically with an almost regular interval, lateralized to one hemisphere and showing no clear evolution in terms of frequency/morphology and amplitude.
Seizures: Sudden, repetitive, evolving and stereotyped ictal pattern with a clear beginning, middle, and ending and a minimum duration of 10 sec.
Status epilepticus: 30 min of seizure activity OR recurrent seizures with no recovery for at least 30 min OR 50% seizure burden in 1 hour period.

32 – 34 weeks

Cyclicity: AS continuous - QS discontinuous
IBI: QS ≤ 10-15 sec
Burst: longer 0.5 - 1.5 Hz, 100 - 200 µV, can have superimposed brushes.
Continuous activity.
Premature temporal theta (PTT) or “temporal sawtooth’’ (runs of rhythmic theta activity of 4.5-6 Hz): disappears in AS at 32 weeks and in QS at 33 - 34 weeks.
Delta Brushes: Decrease in amplitude & increase in frequency (1 to 2 Hz). More numerous at 34 weeks. Occipito-temporal at 32 weeks and occipital at 34 weeks.
Delta: occipital & diffuse in QS.
Theta: diffuse.
Frontal transient (F trans): at 34 weeks; often smooth, incomplete, and asymmetrical.
Positive Rolandic Sharps (PRS): > 0.1 per min.
Positive Temporal Sharps (PTS): 400 ms & > 50 µv & > 0.1 per min.
Sharps (Frontal, Central, Temporal and/or Occipital): > 100 µV & > 0.1 per min.
Mechanical brushes: Spindle-like, fast spiky wave bursts with maximal amplitudes higher than 40uV and frequencies between 13 and 20 Hz.
Deformed delta: Lack of smoothness, wider basis, increased peak-to-peak amplitude.
Immature waves: Presence of waves which are usually seen in previous ages.
Asymmetry: > 50% difference in amplitude and/or frequencies between the 2 hemispheres and/or if pathological waves are exceeding in one hemisphere for > 50% compared to the other.
Asynchrony: Delta waves are mainly synchronous at these ages.
Mild discontinuity: Slightly prolonged IBI OR mildly decreased continuous activity for at least 50% of a 1 hour-period.
Moderate discontinuity: Continuous period of activity > 20 sec less than 10% of the recording (at least 1 hour recording).
Severe discontinuity: Very long IBI, non-reactive OR maximal IBI above one and a half the maximal value for the age group for at least 50% of a 1 hour-period.
Mildly Low voltage: Delta waves < 100 µV (at least 50% of a 1 hour-period).
Moderately Low Voltage: Rare waves between 50 -100 µV, most < 50 µV (at least 50% of a 1 hour-period)
Severely abnormal Low Voltage: persistent ≤ 10 µV (at least 50% of a 1 hour-period).
Isoelectric: Mainly inactive tracing with activity < 5 µV.
Burst suppression: Bursts of theta and/or delta waves (sometimes with fast activity) alternating with periods of low amplitude activity (< 20 µV). No reactivity to stimuli.
Brief Intermittent Rhythmic Discharges (BIRDs): Paroxysms of a seizure-like rhythmic electrographic activity with a duration < 10 sec.
Periodic lateralized epileptiform discharges (PLEDs): Sharp waves or spikes that repeat periodically with an almost regular interval, lateralized to one hemisphere and showing no clear evolution in terms of frequency/morphology and amplitude.
Seizures: Sudden, repetitive, evolving and stereotyped ictal pattern with a clear beginning, middle, and ending and a minimum duration of 10 sec.
Status epilepticus: 30 min of seizure activity OR recurrent seizures with no recovery for at least 30 min OR 50% seizure burden in 1 hour period.

35 – 36 weeks

Cyclicity: AS continuous (at 36 weeks AS 1: high-amplitude continuous tracing; AS 2: continuous, more rapid &lower amplitude) - QS discontinuous.
IBI: QS < 10 sec.
Burst: Longer 0.5 - 1.5 Hz, 100 - 200 µV, can have superimposed brushes.
Continuous activity.
Delta brushes: Both in AS and QS.
Delta: - 1 - 2Hz - Decreased amplitude (100 - 200 µV) - Predominant in occipital during AS; quite diffuse during QS.
Theta: Diffuse.
Slow anterior dysrythmia (SAD): Short bursts monomorphic/polymorphic delta waves, 1 - 3 Hz, amplitude of 50 - 100 µV in frontal areas appears in AS 1.
Frontal transients (F trans): Immature F trans may be repetitive. Mature F trans: sometimes observed at QS onset.
Positive Rolandic Sharps (PRS): > 0.1 per min.
Positive Temporal Sharps (PTS): 400 ms & > 50 µv & > 0.1 per min.
Sharps (Frontal, Central, Temporal and/or Occipital): > 100 µV & > 0.1 per min.
Mechanical brushes: Spindle-like, fast spiky wave bursts with maximal amplitudes higher than 40 µV and frequencies between 13 and 20 Hz.
Deformed delta: Lack of smoothness, wider basis, increased peak-to-peak amplitude.
Immature waves: Presence of waves which are usually seen in previous ages.
Asymmetry: > 50% difference in amplitude and/or frequencies between the 2 hemispheres and/or if pathological waves are exceeding in one hemisphere for > 50% compared to the other.
Asynchrony: Might still be present at the onset of quiet sleep.
Mild discontinuity: Slightly prolonged IBI OR mildly decreased continuous activity for at least 50% of a 1 hour-period.
Moderate discontinuity: Continuous period of activity > 20 sec less than 10% of the recording (at least 1 hour recording).
Severe discontinuity: Very long IBI, non-reactive OR maximal IBI above one and a half the maximal value for the age group for at least 50% of a 1 hour-period.
Mildly Low voltage: Delta waves < 100 µV (at least 50% of a 1 hour-period).
Moderately Low Voltage: Rare waves between 50 - 100 µV, most < 50 µV (at least 50% of a 1 hour-period).
Severely abnormal Low Voltage: persistent ≤ 10 µV (at least 50% of a 1 hour-period).
Isoelectric: Mainly inactive tracing with activity < 5 µV.
Burst suppression: Bursts of theta and/or delta waves (sometimes with fast activity) alternating with periods of low amplitude activity (< 20 µV). No reactivity to stimuli.
Brief Intermittent Rhythmic Discharges (BIRDs): Paroxysms of a seizure-like rhythmic electrographic activity with a duration < 10 sec.
Periodic lateralized epileptiform discharges (PLEDs): Sharp waves or spikes that repeat periodically with an almost regular interval, lateralized to one hemisphere and showing no clear evolution in terms of frequency/morphology and amplitude.
Seizures: Sudden, repetitive, evolving and stereotyped ictal pattern with a clear beginning, middle, and ending and a minimum duration of 10 sec.
Status epilepticus: 30 min of seizure activity OR recurrent seizures with no recovery for at least 30 min OR 50% seizure burden in 1 hour period.

The instructions described each feature clearly and gives very specific guidelines of how to discover certain features. Figure 5-3 shows 14 examples of the different abnormal waveforms/features evident in group 3 and 4.

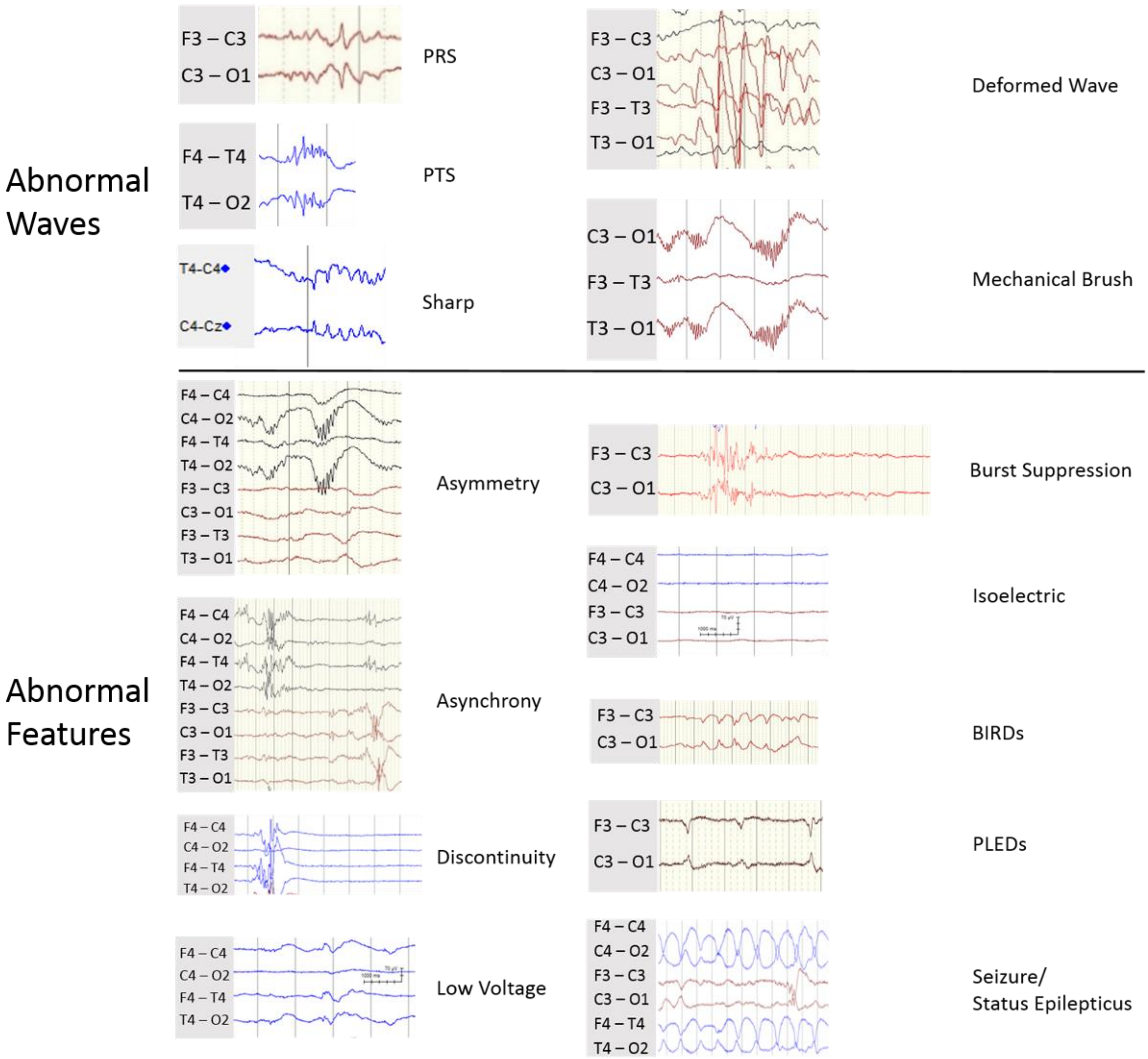

Figure 5-3 Examples of some abnormal waves and abnormal features identified in the assessment scheme.

5.3.4. Third - step analysis

The experts independently scored 12 different infants, (data group 3) (range PMA: 23+3 – 36+1 weeks) EEGs by using the final version of the assessment scheme. EEG time-points of

12 and 24 hours were used for all infants. The clinical characteristics of these infants are in table 5-4.

	Infants Third Step Analysis Median (IQR) (n=12)
GA (weeks)	29.6 (26.3 – 32.2)
BW (g)	1345 (868 – 1985)
Apgar score 5 min	9 (6 – 10)
CRIB II	7 (3 – 12)
Initial pH	7.05 (6.97 – 7.18)
	n (%)
Gender (Male)	5 (42)
IVH Grade III/IV /cPVL	1 (8)
Sepsis	3 (25)
NEC	1 (8)
CLD	2 (17)
ROP	0 (0)
AEDs	2 (17)
Morphine	3 (25)
Mortality	1 (8)

TABLE 5-4 CLINICAL DEMOGRAPHICS OF THE INFANTS INCLUDED IN THE THIRD STEP ANALYSIS. Key: IQR, interquartile range; GA, gestational age; BW, birth weight; g, grams; min, minutes; IVH, intraventricular haemorrhage; cPVL, cystic periventricular leukomalacia; NEC, necrotizing enterocolitis; CLD, chronic lung disease ROP, retinopathy of prematurity; AEDs, anti-epileptic drugs.

Substantial agreement was achieved in 9 out of the 12 neonates, while moderate agreement was obtained in the remaining three infants (Table 5-5). To evaluate the influence of the PMA on the EEG assessment, the patients were subdivided into two age groups (<30 weeks and ≥30 weeks). The older PMAs obtained a better agreement, with a substantial score, while the younger PMAs showed a moderate score. This, however, was not statistically significant.

Patients	Kappa	% agreement	PMA group	Median Percentage Agreement (IQR)	p-value[a]
1	0.783	90			
2	0.500	75			
3	0.566	82.6	**<30 weeks PMA**	82.6 (80.7 to 87.8)	
4	0.704	87			
5	0.649	82.6			
6	0.657	82.6			
7	0.534	78.3			0.249
8	0.725	83.3			
9	1.000	100	**≥30 weeks PMA**	86 (82.1 to 97)	
10	0.669	84			
11	0.746	88			
12	0.909	96			

K values according to Landis:

< 0: no agreement, 0–0.20: slight, 0.21–0.40: fair,0.41–0.60: moderate,0.61–0.80: substantial,0.81–1: almost perfect agreement.

TABLE 5-5 K - SCORES AND PERCENTAGE AGREEMENT FOR INTEROBSERVER AGREEMENT BETWEEN TWO EXPERTS IN PATIENTS' EEG EVALUATION AND CONSIDERING DIFFERENT PMA GROUPS.
[a]Wilcoxon signed-rank test

Furthermore, K-scores and interobserver percentage agreements were calculated for each individual feature (Table 5-6), while the normal and abnormal features groups were also calculated separately. An agreement <50% occurred only once, for the STOPS/Occipital Sawtooth feature group. Retrospective revision of the EEGs and the reviewers analysis identified that a particular EEG was severely pathological, where agreement on the evidence of STOPS/Occipital Sawtooth waves were poor.

Feature Group	Feature	n	% agreement	Kappa
Temporal Organisation	**Cyclicity**	12	83.3	0.429
	Interburst Interval	12	83.3	-
	Burst	12	100	-
	Continuity	10	90	.615
Normal Waves	**STOPS/Occipital sawtooth**	4	25	-
	Delta	12	83.3	-
	Theta	12	91.7	-
	PTT	8	75	-
	Delta Brushes	8	100	-
	Frontal Transient	4	100	1.000
	SAD	2	100	-
Abnormal Waves	**PRS**	12	91.7	0.750
	PTS	12	83.3	0.571
	Sharps	12	75	0.308
	Deformed Waves	12	58.3	0.250
	Mechanical Brushes	12	75	0.438
	Immature Waves	10	70	0.211
Abnormal Features	**Asymmetry**	12	66.7	0.143
	Asynchrony	12	83.3	0.625
	Discontinuity	12	91.7	-
	Low Voltage	12	100	-
	Isoelectric	12	100	-
	Burst Suppression	6	100	-
	BIRDS	12	91.7	0.750
	PLEDS	12	100	1.000
	Seizure	12	100	1.000
	Status	12	100	-

TABLE 5-6 K – SCORES AND PERCENTAGE AGREEMENTS FOR ALL EEG FEATURES.

During comparison of the feature groups, percentage agreement between the 2 experts showed a good agreement for all patients and for each EEG category with median percentage agreement ranging from 80% to 100% across the four categories (Table 5-7). High median values were evident in all feature groups. An agreement <50% occurred only once, where the percentage agreement for normal waves for patient 2 was zero as there was no agreement between reviewers. Retrospective revision of the EEG and the reviewers analysis identified the same severely pathological EEG, as previously mentioned, where one reviewer identified no normal waves at all in the 2 hours epoch and the other identified few normal waves in the background of the abnormal EEG trace. However, both the experts showed a high agreement for this patient in the assessment of the temporal organisation/cyclicity, abnormal waves and abnormal features, showing that they both agreed on the fact that this EEG was clearly abnormal. Apart from this category, the overall agreement for this patient was good.

Patient	PMA Group	Temporal Organisation (%)	Normal Waves (%)	Abnormal Waves (%)	Abnormal Features (%)
1	23-25	100	100	80	88.9
2	23-25	100	0	80	88.9
3	26-27	50	75	83.3	100
4	26-27	75	75	83.3	100
5	28-29	100	100	66.7	77.8
6	28-29	100	100	50	88.9
7	30-31	50	100	66.7	90
8	30-31	100	75	66.7	100
9	32-34	100	100	100	100
10	32-34	100	80	66.7	90
11	35-36	100	80	83.3	90
12	35-36	100	100	83.3	100
Median (IQR)		100 (81 to 100)	90 (75.0 to 100)	80 (66.7 to 83.3)	90 (88.9 to 100)

TABLE 5-7 PERCENTAGE OF AGREEMENT BETWEEN THE TWO EXPERTS FOR ALL FOUR FEATURE CATEGORIES IN EACH PATIENT. Median and inter-quartile range (IQR) were also calculated for each feature category

When the temporal organisation and normal waves were combined to create a normal group and abnormal waves and features combined to create a normal group, high-level of agreement (median 88.9% and 86.6%) between the experts was found. No statistical difference was evident, however, between the percentage agreement between the two groups (p=0.959) (table 5-8).

Patient	PMA Group	Normal Group (%)	Abnormal Group (%)
1	23-25	100	85.7
2	23-25	50	85.7
3	26-27	62.5	93.3
4	26-27	75	93.3
5	28-29	100	73.3
6	28-29	100	73.3
7	30-31	75	81.3
8	30-31	87.5	87.5
9	32-34	100	100
10	32-34	88.9	81.3
11	35-36	88.9	87.5
12	35-36	100	93.8
Median (IQR)		88.9 (75 to 100)	86.6 (81.3 to 93.3)
Difference: Median (IQR)		0.70 (-15.3 to 12.6)	
p-value[a]		0.959	

TABLE 5-8 PERCENTAGE AGREEMENT BETWEEN THE TWO EXPERTS FOR NORMAL AND ABNORMAL FEATURES IN EACH PATIENT. Median (IQR) were also calculated for each feature category. Wilcoxon signed-rank test was used to compare the performance of normal and abnormal features. [a]Wilcoxon signed-rank test

5.4. Discussion

We present a tailored, age-specific preterm EEG assessment scheme with user instructions, to specifically evaluate the EEG of preterm infants at different PMA, summarizing all the current knowledge about this topic. The six different age groups were chosen accordingly to the existing literature (175) that provided this subdivision following the evolution of the EEG features. Groups of approximately 2 weeks is usually accepted in the estimation of the GA by EEG visual analysis, and was viewed as the best method to implement in the development of the scheme. The selection of what was included was carefully considered, to make the scheme as concise and as user friendly as possible to aid analysis at the cot side. However, of course, the time required to use the EEG assessment scheme may vary, depending on the reviewer expertise and difficulty of the EEG trace, being largely subjective. The main usefulness of this scheme is that it provides a defined list of all the EEG features that needs to be assessed at each PMA in order to accomplish an objective revision; therefore, this scheme reduces the reviewers' subjectivity guiding their assessment. This scheme enables qualitative assessment of the EEG, however it also enables further quantitative analysis, if required.

This scheme is sub-divided into 6 PMA groups and also 4 EEG categories: temporal organisation, normal waves, abnormal waves and abnormal features. Using this method, we demonstrate moderate to almost perfect interobserver agreement between two independent experts from two different centres, who were not involved in the development of the scheme. Between the experts, the median percentage agreement rates for all four categories of EEG features were between 80% and 100%, while the mean percentage agreement for normal features was 89% and the abnormal features were 87%, with no significant difference in agreement between normal and abnormal groups ($p=0.959$). This provides a plausible start-point for this scheme to be considered as a useful tool for EEG assessment in preterm infants.

Examiners' expertise and effective quality of training given on the newly developed assessment scheme is important to ensure high percentage interobserver agreement of EEG data interpretation (403). Visual analysis of EEG shows different interobserver agreement at different postnatal ages and when considering different EEG features in adults and older children (411, 412). Epileptic discharges have specifically shown almost perfect/substantial agreement between experts, whilst focal non-epileptic abnormalities have shown moderate results in children with new diagnosis of seizure (411). Additionally, it has been demonstrated that using precise definitions during EEG analysis in children, might improve the interobserver agreement (411).

A recent study for neonatal EEG has shown variability in neonatal EEG background interpretation across electroencephalographers (413). Interrater agreement was consistently highest for voltage, seizure presence, continuity, burst voltage, suppressed background presence, delta activity presence, theta activity presence, and overall impression. However, agreement was poor or inconsistent for other features such as burst abnormality type and interburst voltage. Due to the peculiarities of neonatal EEG, we believe that the use of well-defined EEG features and a shared assessment scheme would be beneficial in order to have an objective qualitative analysis of the neonatal EEG and a better agreement between observers for preterm EEG evaluation.

Some studies testing interobserver agreement for neonatal seizure detection in term infants (269, 414, 415). Interobserver agreement was high when international neonatal EEG experts reviewed multichannel EEG for seizure detection; however, lower agreement was reported in shorter or rarer seizures (414). In another study, the authors reported a substantial interobserver agreement when two experts reviewed 2-channel continuous EEG with aEEG for seizure detection in term infants, compared to a fair degree of agreement when only using the aEEG (269). The most recent study reported suboptimal reliability, even when experienced clinicians used aEEG to detect seizures. Video was available during the investigation, however this did not seem to improve the aEEG recording analysis (415).

However, little is known about interobserver agreement in neonatal EEG interpretation in general, particularly for background activity evaluation and for its assessment in preterm infants, for whom only intermittent features have been studied (416). Murphy et al. calculated interobserver agreement was only calculated for burst/IBI detection and showed that moderate levels of agreement (median Kappa from 0.53 to 0.66) were achieved among 3 observers with annotations across all channels (416). A recent paper, with annotations on a channel-by channel basis, achieved a Kappa score agreement of 0.60 (95% CI: 0.21 to 0.74) (417).

A comprehensive glossary for neonatal EEG has previously been developed by André et al. (175). Recently, a standard computer-based system for EEG assessment and reporting has been developed with a subsection on neonatal EEG for both term and preterm infants (403, 418). Additionally, previous studies gave indications on normal and abnormal features of the EEG in preterm (174, 277). However, because of the increasing survival rates of very and extremely preterm infants, knowledge about the features of the EEG in this population is still growing, particularly with respect to the normal duration of IBI at specific GA/PMA. This could potentially be the reason why the highest disagreement between raters occurred in the lower ages and for the abnormal waves. Thus, uncertainties about boundaries between the normal and the abnormal features are still present and differences exist between EEG readers and different centres. A tailored scheme for infants at different PMAs and normative definitions for normal and abnormal features are needed in order to correctly grade the preterm EEG. Certainly, there is a huge demand to assess maturation of brain function, how to effectively monitor and manage brain development and how to correctly assess prognosis. A defined EEG assessment scheme to facilitate the analysis of preterm EEG is still lacking and should be addressed.

EEG background activity and the presence of seizures have already been shown to be related to outcome in preterm infants (194, 288, 289, 307, 308, 314, 321, 325, 395, 404-408, 419). Thus, multichannel EEG is a valid tool to assess preterm neurodevelopment. Its usefulness depends on the experience of the reader, thus providing an objective method to evaluate and grade EEG in preterm infants is warranted.

Previously developed neonatal EEG scoring systems were based on mixed populations of both preterm and term infants (194, 402), however, they were not tailored on the growing population of very and extremely preterm infants. Here, a scheme and a user instructions specifically for EEG evaluation in preterm infants divided in different PMAs is developed, summarizing all the current knowledge. Using this method, the inter-rater agreement between electroencephalographers were very good, however this agreement may be biased since we developed the scheme. Alternatively, two independent experts from different centres produced good inter-rater agreement, with moderate agreement for all patients, PMAs and EEG features. Slight bias is a possibility at this stage also, since the experts discussed the features before finalising the scheme. A slightly better result is obtained for older PMAs (≥ 30 weeks), possibly reflecting the better knowledge available for this age group, however, the difference is not substantial. In terms of the features, the kappa scores were not always measureable, due to the lack of variability in the scorings between the raters. Percentage agreement was therefore calculated, with high agreements (>50%) in all but one feature, which was the STOPS/occipital sawtooth feature. In terms of the four feature groups, all four produced high median percentage agreement values, ranging from 80 – 100%, while additionally further grouping of normal and abnormal groups produced high median percentage agreement of 88.9% and 86.6%, respectively. The difference in median was not statistically significant between the normal and abnormal groups. Therefore, we believe that this approach offers a higher possibility to achieve a consensus in the evaluation of the preterm EEG between different readers and lays the foundation for a tailored assessment scheme of preterm EEG for prognostic purposes in this population. A limitation of the present study is the number of subjects evaluated, accordingly we advise future studies to use a larger sample size. Future objectives should be to implement the present scheme for grading preterm brain function in a large sample of preterm infants. Additionally, it is important to assess their performance for serial follow-up EEGs and for the prediction of neurodevelopmental outcome. Eventually, it might be possible to evaluate the performances of the final developed assessment scheme in other research centres.

In conclusion, this work presents the first step towards a standardized scheme for the analysis of EEG in preterm infants. This will allow a better understanding of the relationship between EEG and prognosis in this population. Clear definitions and identification of features will possibly improve user confidence during pattern recognition in preterm EEG. This is an attempt to simplify and provide clarity to EEG readers, identifying the features they need to take into account when approaching a preterm EEG. The hope is that this scheme provides clarity and is user friendly, ultimately allowing a higher interobserver agreement. If the approach is used, it may be possible to easily compare studies performed in different centres in the near-future. This scheme allows for a more universal way of assessing preterm EEG and the opportunity to pool larger data sets. The reported high interobserver agreement between experts is a promising sign for the ability to assess the preterm infant EEGs in the future, and ultimately being of a clinical use in the early neonatal period.

Chapter 6. Mathematical and visual analysis of serial EEG concordance in preterm twin infants

Part published as:

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6.1. Introduction

Multiple births constitute 2.4 – 4% of all live births, of which 42 – 78% are preterm infants below 37 weeks GA (420). Preterm twins are at an increased risk of adverse outcome, such as CP (421) with monochorionic-diamniotic (MCDA) twins at higher risk of mortality and morbidity compared to dichorionic-diamniotic (DCDA) twins (422). In addition, the early birth of very and extremely preterm infants leads to added complications (423). A study by Bodeau-Livinec et al showed that twins <32 weeks GA had higher mortality and slightly lower neurodevelopmental outcome scores at 5 years of age compared to preterm singletons (424).

EEG is the gold standard for accurate monitoring of cerebral activity and its portability makes it particularly suited to the NICU. It can accurately predict long-term outcome from the first few days of life (293, 306, 307, 309, 321). EEG characteristics are believed to be strongly influenced by genetics (425). Studies have reported EEG similarities in child and adult twins (426, 427) with greater similarities for identical twins compared to non-identical twins (428-432). Similar findings have been reported in term neonates, with identical twins showing stronger correlations compared to non-identical twins (433, 434).

To our knowledge, no EEG studies in preterm twins have been conducted, therefore it remains unclear whether this concordance starts before term age. We therefore aim to determine if EEG concordance exists for twins born <32 weeks GA and determine if this concordance, if present, is dependent on twin type (MCDA and DCDA). We also aim to assess the effect of maturation on concordance over the neonatal period.

6.2. Material and Methods

6.2.1. Participants

This is a retrospective study of preterm twins that were selected from a large prospective cohort study of infants <32 weeks GA, born in Cork University Maternity Hospital Ireland and enrolled between March 2013 and April 2014. To assess whether non-related preterm infant pairs had similar EEG concordance to preterm twin pairs, singleton infant pairs were selected from the prospective study. The singletons were matched on GA to the twin pairs. Any infant with IUGR, grade 3 or 4 IVH, congenital anomaly, or who died during the NICU stay, was excluded from this study. Ethical approval for the collection and analysis of the data was granted by the Clinical Research Ethics Committee of the Cork Teaching Hospitals, Ireland and written informed parental consent was obtained.

6.2.2. Data collection

Clinical details and infant demographics were recorded. Twin type information was obtained from the ultrasound examination during pregnancy. MCDA twins were distinguished as fetuses with a shared chorionic sac and individual amniotic sacs whereas in DCDA twins each fetus had an individual chorionic and amniotic sac (29). Results from serial CRUS scans were collected and documented for all infants, including information regarding grade of IVH, to ensure exclusion of the infants with severe IVH. As per our standard clinical practice, a paediatric radiologist, who was not involved in the study, performed and reported all CRUS scans. Infants had the first CRUS within the first 72 hours of birth where possible, with repeat scans between 7 – 10 days of age and again at one month. Timings varied slightly depending on the availability of the radiologist and the infants' clinical condition. For each infant, we calculated the CRIB II, a clinical assessment with possible scores ranging from 0 to 27 (42). This instrument calculates an index of risk based on gender, GA, BW, admission temperature, and the base deficit in the first blood sample taken.

6.2.3. EEG Recording

Continuous video-EEG was recorded for all infants, after birth, as soon as they were stable. Recordings for twin pairs started at almost the same time and continued until the infants were approximately 72 hours of age; some recordings were continued for longer to accommodate clinical care or parental visitation. EEGs, of 2-4 hours duration, were repeated at 32- and 35-weeks PMA. All multichannel EEG recordings also displayed a 2 channel aEEG simultaneously on the EEG monitor. A modified neonatal version of the international 10/20 system of electrode placement was used (344). Disposable Ambu Neuroline 700 Single Patient Surface Electrodes were applied to the scalp. Active electrodes were positioned at F4, F3, C4, Cz, C3, T4, T3, O2 and O1, with reference electrode at Fz and ground electrode behind the left ear. In some instances, the Cz electrode was not applied because of small head size. Impedances below 5kΩ were maintained throughout the recording. Three EEG machines were available for use during the recruitment period: the NicoletOne™ EEG system (CareFusion Co., San Diego, USA); the Nihon Koden, EEG-1200, Neurofax; and the Moberg ICU Solutions, CNS-200 EEG and Multimodal Monitor (344). Continuous multichannel EEG was recorded and analysed by research staff and the aEEG was available to aid clinical management. Concerns about clinical behaviours or aEEG patterns during monitoring led to review of the continuous multichannel EEG, but this was dependent on the availability of a neurophysiologist.

6.2.4. Visual EEG analysis

The EEG recordings were visually analysed for quality and, if this was poor, the infants were excluded. Standard visual analysis of the entire EEG recording in each infant was undertaken to assess for seizure activity, state change, maturational features such as delta brushes, and abnormal features such as deformed waveforms or disorganised waveforms (194).

Two-hour EEG epochs with zero to minimal artefact at 12, 24, 48, 72 hours post-natal age, and 32 weeks and 35 weeks PMA were extracted from the recordings. The first four epochs, within 72-hours post-natal age, were grouped together and henceforth referred to as the early time-point. Therefore, over the infant’s stay in the NICU, three time-points were analysed: early, 32 weeks and 35 weeks. The 32 week recording was chosen as a significant time-point as it represents a milestone age with decreased risk of morbidity and mortality (435) and the 35 week recording was chosen as a pre-discharge recording. For most infants, epochs at all time-points were available, but some were missing due to late EEG application, instability of the infant, poor quality of the EEG recording at that time period, or early discharge.

To avoid bias during visual analysis, epochs from all infants were de-identified and ordered randomly. The only remaining information available during the EEG analysis period was the GA of the infant, and any medication given at the time of recording. The infant’s identification code was reinstated following analysis. Visual analysis was performed according to the assessment scheme in Chapter 5 (Figure 5-2, page 170). This approach was used on two-hour epochs from each infant. Figure 5-2 lists all features assessed at each GA/PMA. In total, 15 EEG features were assessed in the 23 – 25 GA age group; 18 EEG features in the 26 – 27 GA and 28 – 29 GA age group; 19 EEG features in the 30 – 32 GA age group; and 20 EEG features were studied in the 35 – 36 GA age group. This assessment criteria in Figure 5-2 was developed following an extensive review of the existing literature in order to create a scheme of EEG evaluation in very and extremely preterm babies at different GAs (174, 175, 194, 277, 292, 309, 402, 409, 410).

Features were graded as either 1 or 0 to indicate the presence or absence of that feature. Thus a binary sequence corresponding to the number of features was associated with each epoch.

6.2.5. Mathematical EEG analysis

We extracted a set of mathematical features from each EEG epoch using the NEURAL software package (436). Although the epochs had minimal artefact, the first stage of the feature extraction procedure was a simple artefact detection algorithm to remove any remaining artefacts such as brief high-amplitude transients. Next, the EEG was low-pass filtered to 30 Hz and down-sampled from 256 Hz to 64 Hz. Mathematical features were then extracted using the bipolar montage F4–C4, F3–C3, C4–T4, C3–T3, C4–Cz, Cz–C3, C4–O2, and C3–O1. For full details on the artefact removal procedure and feature estimation methods, please see (436).

EEG features were categorized into power, discontinuity, and symmetry groups. The power group consists of absolute and relative spectral power. Relative spectral power was calculated as the power in each frequency band relative to the total power over the 4 bands. The discontinuity group consists of kurtosis and skewness of the EEG and features that represent the temporal organization of the bursts, namely percentage of bursts and median inter-burst interval. Bursts were detected using an automated burst detection method (417). The symmetry group consists of inter-hemispheric coherence. All features, except for the burst features, were calculated on four frequency bands: 1) 0.5 to 3 Hz; 2) 3 to 8 Hz; 3) 8 to 15 Hz;

and 4) 15 to 30 Hz. These modified frequency bands better suit the distribution of energy in preterm EEG (208, 436). Spectral power and coherence features used the Welch method to estimate the power spectral density, using a 2-second Hamming window with 50% overlap. For calculating the inter-hemispheric coherence, we used the surrogate-data approach to estimate zero coherence using 1,000 surrogates of random-phase signals with a 95-percentile cut off (436).

All features, again except for the burst features, were extracted from short-duration segments (64 seconds with a 50% overlap) over the whole 2-hour epoch. The median value over all segments was used to summarize the feature for the 2-hour epoch, which is the median value over approximately 225 segments. All features, excluding the symmetry features, were summarised by the median value over channels. In total, 22 features were generated: eight features for power, 10 features for discontinuity, and four features for the symmetry group.

6.2.6. Statistical Analysis

Continuous data were described using the median and IQR and categorical data with number and percentage. All tests were two-sided and a p <0.05 was considered statistically significant.

6.2.6.1. Visual Analysis

To evaluate concordance using the visual EEG grading procedure, Pearson's correlation coefficient (r) was calculated for each binary feature sequence within twin pairs at all time-points. For the early time-point, four r-values from the first 72 hours were averaged to obtain one r-value representative of this time point. This procedure was repeated for the age-matched singleton pairs. The strength of the coefficient values was determined using Cohen's guidelines : r of 0.1 – 0.3 (small correlation), 0.3 – 0.5 (moderate correlation), >0.5 (strong correlation) (437). Correlation coefficients were then compared between the twins and singletons. For all 3 time-points, differences in the correlation distributions were tested using the Wilcoxon signed- rank test when the data was paired (twins vs singletons) and the Mann-Whitney U-test when the data was independent (MCDA vs DCDA).

6.2.6.2. Mathematical Analysis

To evaluate concordance for the mathematical EEG analysis, we used intra-class correlation (ICC) to assess the differences within twin-pairs compared to among all twins, with higher ICC values indicating greater similarity for twin-pairs. ICCs were calculated for all twins, then all singletons, and then MCDA and DCDA sub-groups. We used linear mixed-effect models to estimate the ICCs. Due to non-Gaussian skew in the distributions of many features, all features were log transformed before analyses. The ICC was defined as the proportion of variation of the feature that was due to the variance in the twin effects (438). ICCs for later time-points (32 weeks and 35 weeks) were estimated, with twin group as a random effect in the model, as : ICC (1 epoch) = $\frac{\sigma_{twin}^2}{\sigma_{twin}^2+\sigma_{residual}^2}$, where σ_{twin}^2 represents the variance within the twin-pairs and $\sigma_{residual}^2$ represents the variance of the residual from the model fit. ICCs for the early time-points (which include the 4 epochs at <72 hours post-natal age) were estimated, with both individual and twin group as random effects, as: ICC (4 epochs) $=\frac{\sigma_{twin}^2}{\sigma_{twin}^2+\sigma_{individual}^2+\sigma_{residual}^2}$, where $\sigma_{individual}^2$ represents the variance among all twins. As GA may significantly alter twin correlations (439), adjusted ICCs were estimated by including GA as a fixed effect in the models. Both unadjusted and adjusted ICCs are reported.

The small size of our cohort may bias our estimate of ICC values. To ensure confidence in the estimate, we implemented a method to test if the ICC significantly differs to 0. We applied an approach used for assessing coupling in EEG, often referred to as the surrogate-data approach (440). The method generates a probability distribution associated with the null hypothesis that the ICC is zero, to generate a threshold above which significantly differs to zero. To calculate this distribution, we generated surrogate data for every ICC estimate. First, random samples are generated from a Gaussian distribution, with the same mean and standard deviation as the log of the feature. Then, these random samples are used to generate an ICC using the same number of infants and twin pairings. This process is repeated 1,000 times resulting in a null-hypothesis distribution of ICC values (441). The lower threshold is estimated from the 95th percentile of this distribution. And finally, if the ICC of the feature is greater than this threshold then we infer that the ICC is significantly different to 0 (p <0.05). This process was repeated for all features at all three time-points in the analysis.

The strength of agreement (ICC values) was interpreted according to the guidelines of Landis and Koch: ICC of 0 – 0.2 (slight), 0.2 – 0.4 (fair), 0.4 – 0.6 (moderate), 0.6 – 0.8 (substantial), 0.8 – 1 (almost perfect agreement) (442). For this analysis, we define significant ICCs as values above 0.6 that exceed the threshold from the surrogate data. The ICCs were calculated using R (version 3.3.1, The R Foundation of Statistical Computing, http://www.r-project.org) with the *lme4* package (version 1.1-10). Mathematical EEG features were generated using version 0.3.0 of the NEURAL software package. All other statistical analyses were performed in SPSS Statistics 21 (SPSS Inc, Chicago, Illinois).

6.3. Results

6.3.1. Patient characteristics

In total, 36 preterm infants (20 twin individuals and 16 singletons) were eligible for this study. During the recruitment period, 67 infants born less than 32 weeks GA were enrolled, of which 13 sets of preterm twins (26 infants) were monitored. This included six sets of MCDA twins (12 infants) and seven sets of DCDA twins (14 infants). From the 13 twin sets, three were excluded: one due to congenital anomalies, and two due to IUGR, IVH grade III/IV and death during hospital stay. No EEGs were excluded due to poor quality recordings. Consequently, 10 sets of twins (4 MCDA pairs and 6 DCDA pairs) were included in the study. Median duration of the EEG recordings at the different time-points and the postnatal age at the start of the first EEG recording are evident in Table 6-1.

	Twins Median (IQR)		Singletons Median (IQR)	
Duration early EEG (hrs)	*(n=20)*	69.4 (65.8 to 71.7)	*(n=16)*	69.4 (62.4 to 71.4)
Duration 32wks EEG (hrs)	*(n=19)*	3.2 (2.6 to 5.2)	*(n=16)*	2.7 (2.0 to 4.2)
Duration 35wks EEG (hrs)	*(n=16)*	2.4 (2.0 to 3.6)	*(n=11)*	3.0 (2.3 to 3.2)
Postnatal Age at start of first EEG (hrs)	*(n=20)*	7.8 (6.1 to 9.8)	*(n=16)*	7.3 (3.5 to 12.8)

TABLE 6-1 EEG RECORDING COMPARISONS BETWEEN TWINS AND SINGLETONS, INCLUDING THE DURATION OF EEG RECORDINGS AT DIFFERENT TIME-POINTS AND POSTNATAL AGE AT START OF THE FIRST RECORDING.

In total, 210 two-hour EEG epochs were visually reviewed before being mathematically analysed for both twin pairs and control age-matched singleton pairs. From the group of twins, 20 infants were recorded during the early time-point, 19 were recorded at 32 weeks and 16 were recorded at 35 weeks. Within our cohort we found 16 eligible singleton infants to generate the 10 unique age-matched singleton pairings. This meant that 4 infants were reused, but paired with different singletons to create new singleton pairings. From the 10 singleton pairings, 20 unique EEGs pairings were used during the early time-point, 20 EEG pairs were used at 32 weeks and 14 EEG pairs were used at 35 weeks. Table 6-2 describes the clinical demographics of the MCDA, DCDA twins and singleton infants.

		MCDA twins (n=8)	DCDA twins (n=12)	Singletons (n=16)
Gestational Age (weeks)	median (IQR)	30.6 (27.8 – 31.3)	30.3 (29.1 – 31)	30.5 (27.9 – 31.3)
Birth weight (g)	median (IQR)	1285 (948 – 1650)	1635 (1368 – 1775)	1430 (1148 – 1548)
Gender (Male)	n (%)	6 (75)	8 (67)	9 (56)
5-min Apgar score	median (IQR)	9 (8 – 9.5)	9 (9– 9.8)	9 (7.25 – 9)
First pH	median (IQR)	7.22 (7.16 – 7.25)	7.19 (7.17 – 7.22)	7.24 (7.16 – 7.33)
CRIB II score	median (IQR)	3.5 (2.5 – 8.25)	3.5 (2.25 – 5)	4.5 (2 – 8)
Infants on Morphine	n (%)	2 (25)	1 (8)	3 (19)
Individuals with IVH I/II	n (%)	1 (12)	5 (42)	5 (31)

TABLE 6-2 CLINICAL DEMOGRAPHICS OF MCDA, DCDA TWINS AND SINGLETONS. Key: MCDA, monochorionic-diamniotic; DCDA, dichorionic-diamniotic; IUGR, intrauterine growth restriction; IVH, intraventricular haemorrhage.

6.3.2. Visual EEG Analysis

It was evident that some mild abnormalities in cyclicity, IBI and bursts occurred in four of the six infants that had mild CRUS abnormality (IVH I-II). Deformed waveforms (Figure 6-1) were common features that appeared in the six infants with mild IVH injury, although these waveforms were also occasionally evident in infants with normal CRUS. Specifically, however, at 35 weeks, deformed waveforms were only evident in infants with grade I/II IVH. Deformed waveforms are regarded as high amplitude delta brush activity with spiky, cogwheel-shaped appearance that lack smoothness.

TWIN A

TWIN B

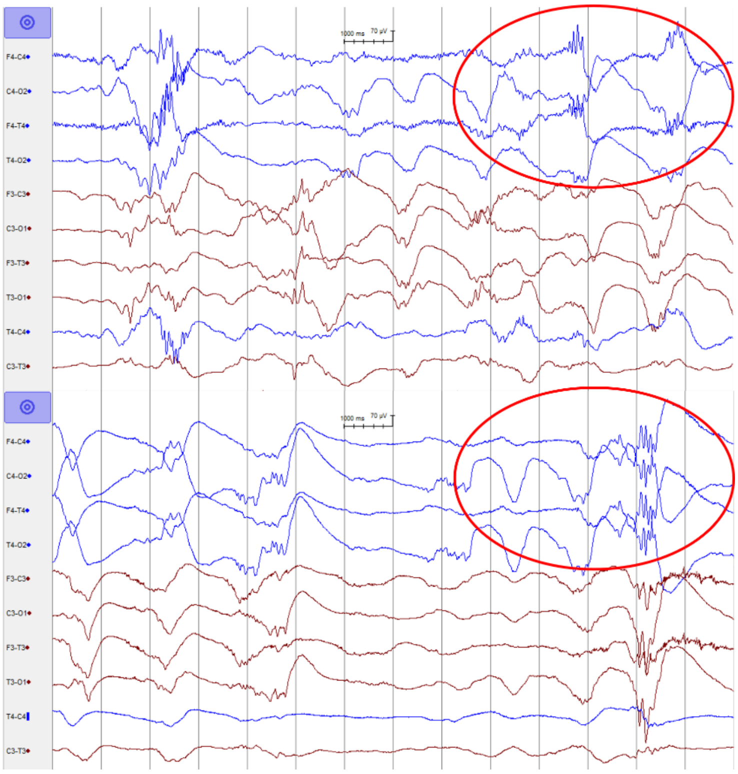

Figure 6-1 Synchronised EEG of MCDA twin pair 2 (30+0 week GA) at 13 hours of age. Evidence of deformed waves maximally over the right hemisphere in both twins.

Table 6-3 shows no difference in correlation values between twin pairs and age-matched singleton pairs for the three time-points (*p* >0.05 for all). Strong *r*-values (r >0.5) are evident in both the twins and the singletons at all time-points.

	Twins Median Correlation (IQR)	**Singletons Median Correlation (IQR)**	***p*-value [a]**
Early	0.72 (0.58 – 0.78)	0.79 (0.64 – 0.83)	0.386
32wks	0.83 (0.83 – 0.83)	0.69 (0.64 – 0.94)	0.397
35wks	0.83 (0.69 – 0.83)	0.83 (0.74 – 0.87)	0.180

TABLE 6-3 CORRELATION VALUES BETWEEN TWIN AND SINGLETON PAIRS.

[a] = Wilcoxon Signed Ranked Test

Table 6-4 provides the correlation results within each twin pair. Strong *r*-values (*r*>0.5) are evident in all twin pairs for all time-points. We found no statistical significant difference between the MCDA and DCDA groups at each time-point (*p* = 0.670 – 1.00).

	GA	IVH (Grade)	**Early**	**32 weeks**	**35 weeks**
Twin pairs			*r*	*r*	*r*
MCDA 1	27+0	One infant (II)	0.51	1.00	0.83
MCDA 2	30+0		0.57	0.56	0.69
MCDA 3	31+2		0.87	0.83	0.83
MCDA 4	31+1		0.73	0.83	0.83
MCDA twins – Median (IQR)			0.65 (0.55 – 0.76)	0.83 (0.76 – 0.87)	0.83 (0.79 – 0.83)
Twin pairs			*r*	*r*	*r*
DCDA 1	26+1	Both infants (II)	0.83	NA	1.00
DCDA 2	31+0	Both infants (I)	0.60	0.83	NA
DCDA 3	30+2	One infant (II)	0.53	0.83	0.83
DCDA 4	31+6		0.79	0.83	NA
DCDA 5	30+2		0.78	0.83	0.69
DCDA 6	29+1		0.72	0.83	0.69
DCDA twins – Median (IQR)			0.75 (0.63 – 0.78)	0.83 (0.83 – 0.83)	0.76 (0.69 – 0.87)
***p*-value** [a]			***0.670***	***1.00***	***0.874***

TABLE 6-4 CORRELATION VALUES OF WITHIN THE TWIN PAIRS AT THREE DIFFERENT TIME-POINTS. Key: r, correlation values; MCDA, monochorionic-diamniotic; DCDA, dichorionic-diamniotic; NA indicates missing data.

[a] = Mann-Whitney U test

Three of the four twin pairs, with mild CRUS abnormalities have three of the lowest *r*-values at the early time-point. These low values are only evident at the early recording and increased by 32 and 35 weeks. The fourth twin pair with mild IVH had the highest *r*-values, with both individuals showing the same brain injury grade.

6.3.3. Mathematical EEG analysis within twin pairs

Table 6-5 provides adjusted ICCs for all the twins, the MCDA and DCDA types separately and also the singletons for all mathematical features at the three different time-points. Table 6-6 provides unadjusted ICCs for comparison purposes. Adjusting for GA decreased the majority of ICCs at the early time-point. For the twins, five significant (>0.6 with $p<0.05$) ICC values are evident at the early time-point, four at 32 weeks, and seven at 35 weeks after adjusting for GA. Eight significant ICCs are evident in the discontinuity category, six in the power category, and two in the symmetry category. For the control singleton analysis, we found no significant ($p>0.05$ or ICC≤0.6) ICCs after adjusting for GA.

			Adjusted ICC values											
Feature Category	Quantitative Feature	Freq Band	All Twins			MCDA			DCDA			All Singletons		
			Early	32w	35w	Early	32w	35w	Early	32w	35w	Early	32w	35w
1. Power	Absolute Spectral Power	1	0.43	-	-	0.45	-	-	0.40	-	-	0.25	-	-
		2	0.51	0.60	**0.61***	**0.88***	**0.77***	**0.75***	0.25	-	-	-	-	-
		3	0.39	**0.74***	**0.75***	**0.80***	-	**0.90***	0.22	**0.79***	-	-	-	-
		4	-	**0.68***	-	0.39	-	**0.76***	-	-	-	0.43	-	-
	Relative Spectral Power	1	0.28	-	-	0.44	-	-	-	-	-	0.48	-	-
		2	0.29	-	-	0.52	-	-	-	-	-	0.37	-	-
		3	0.43	**0.75***	-	-	-	-	0.27	-	-	0.29	-	-
		4	0.48	**0.76***	-	0.43	-	-	0.29	**0.73***	-	0.34	-	-
2. Discontinuity	Amplitude Skewness	1	0.43	-	-	**0.76***	-	-	-	-	-	-	-	-
		2	0.30	-	0.59	**0.61***	**0.81***	**0.76***	-	-	-	-	-	-
		3	**0.65***	0.56	**0.71***	**0.83***	**0.93***	**0.89***	0.24	-	-	-	-	-
		4	**0.69***	-	-	**0.81***	-	-	0.22	-	-	-	-	-
	Amplitude Kurtosis	1	**0.62***	-	-	**0.90***	-	-	-	-	-	-	-	-
		2	**0.62***	-	**0.71***	**0.85***	**0.93***	**0.81***	0.23	-	-	-	-	-
		3	0.60	-	-	**0.83***	**0.88***	**0.76***	-	-	-	-	-	-
		4	**0.61***	-	-	**0.77***	-	-	-	-	-	-	-	-
	IBI Length (median)		0.59	0.55	**0.70***	**0.76***	**0.83***	-	0.32	-	-	-	-	-
	Burst Percentage		0.57	-	-	**0.66***	-	-	-	-	-	-	-	-
3. Symmetry	Hemisphere Coherence	1	0.14	-	**0.79***	-	-	**0.86***	-	-	-	-	-	-
		2	0.50	-	**0.84***	**0.74***	-	**0.96***	-	-	-	-	-	-
		3	0.53	-	-	**0.79***	-	**0.96***	-	-	-	-	-	-
		4	0.47	-	-	**0.72***	-	-	0.22	-	-	-	-	-
Number of significant ICCs			16 significant ICCs from 13 features (>0.6 with *p* <0.05)			31 significant ICCs from 17 features (>0.6with *p* <0.05)			2 significant ICCs from 2 features (>0.6 with *p* <0.05)			0 significant ICCs (>0.6 with *p* <0.05)		

TABLE 6-5 ADJUSTED FOR AGE INTRA-CLASS CORRELATION (ICC) VALUES OF ALL TWIN INFANTS, MCDA INFANTS, DCDA INFANTS AND CONTROL SINGLETONS AT THE EARLY, 32 WEEK AND 35 WEEK TIME-POINTS.Highlighted (bold and asterisk) values if ICC is significant and substantial (i.e. >0.6 and p*<0.05). Non-significant values (p≥0.05) are omitted. Analysis controls for gestational age; unadjusted values in supplementary material.*

			Unadjusted ICC values											
Feature Category	Quantitative Feature	Freq Band	All Twins			MCDA			DCDA			All Singletons		
			Early	32w	35w	Early	32w	35w	Early	32w	35w	Early	32w	35w
1. Power	Absolute Spectral Power	1	0.62	-	-	0.63	-	-	0.60	-	-	0.22	0.83	-
		2	0.67	0.56	0.58	0.89	0.75	-	0.50	-	-	-	-	-
		3	0.40	0.71	0.73	0.78	-	0.86	0.16	0.81	-	0.16	-	-
		4	0.05	0.65	-	0.29	-	-	-	0.72	-	0.55	-	-
	Relative Spectral Power	1	0.31	-	-	0.34	-	-	0.27	-	-	0.43	-	-
		2	0.26	-	-	0.50	-	-	0.20	-	-	0.35	-	-
		3	0.62	0.73	-	-	-	-	0.69	0.81	-	0.29	-	-
		4	0.74	0.77	-	0.62	-	-	0.75	0.83	-	0.62	-	-
2. Discontinuity	Amplitude Skewness	1	0.66	-	-	0.79	-	-	0.55	-	-	-	-	-
		2	0.64	-	0.54	0.72	-	-	0.59	-	-	-	-	-
		3	0.78	-	0.67	0.85	0.90	0.84	0.71	-	-	0.17	-	-
		4	0.80	-	-	0.84	-	-	0.73	-	-	-	-	-
	Amplitude Kurtosis	1	0.71	-	-	0.88	-	-	0.48	-	-	-	-	-
		2	0.76	-	0.67	0.87	0.90	0.73	0.62	-	-	0.23	-	-
		3	0.73	-	-	0.86	0.83	-	0.53	-	-	-	-	-
		4	0.71	-	-	0.77	-	-	0.55	-	-	-	-	-
	IBI Length (median)		0.59	0.51	0.67	0.72	0.80	-	0.30	-	0.78	-	-	-
	Burst Percentage		0.56	-	-	0.57	-	-	0.18	-	-	-	-	-
3. Symmetry	Hemisphere Coherence	1	0.10	-	0.77	-	-	0.81	-	-	0.76	-	-	-
		2	0.55	-	0.84	0.66	-	0.94	0.39	-	-	0.32	-	-
		3	0.62	-	-	0.74	-	0.94	0.49	-	-	0.36	-	-
		4	0.47	-	-	0.63	-	0.56	0.23	-	-	-	-	-

TABLE 6-6 UNADJUSTED FOR AGE INTRA-CLASS CORRELATION (ICC) VALUES OF ALL TWIN INFANTS, MCDA INFANTS, DCDA INFANTS AND CONTROL SINGLETONS AT THE EARLY, 32 WEEK AND 35 WEEK TIME-POINTS. Non-significant values (p≥0.05) are omitted.

The MCDA twins had 31/66 significant correlations (ICC>0.6; p<0.05) for 17 features across all feature groups: 17 almost perfect (>0.8) correlations and 14 substantial (>0.6) correlations (see Table 6-5). In contrast, the DCDA group had only 2/66 significant correlations (ICC>0.6; p<0.05), with both being substantial ICCs. For the MCDA twins, three features (spectral power at 3 – 8 Hz, skewness at 3 – 15 Hz, and kurtosis at 3 – 15 Hz) had significant ICCs over the course of all three time-points. No features for the DCDA group had significant ICCs over all three time-points.

For the discontinuity features in the MCDA group, there were 19/30 significant correlations (12 almost perfect and seven substantial) across all time-points: all 10/10 at the early time-point and 9/20 for the later time-points. Half (6/12) of the spectral power measures had significant correlations, but not in the delta (0.5–3 Hz) range; no significant correlations were found for relative spectral power at any of the time-points. The highest ICCs (0.96) were found in the inter-hemisphere coherence measures at the 35-week time-point in the middle frequency range 3–15 Hz.

6.4. Discussion

Our findings confirm that preterm twins exhibit EEG concordance during the early neonatal period in the NICU, suggesting a strong genetic influence on the EEG from an early age. Mathematical EEG analysis shows substantial/almost perfect ICCs between the twin pairs, particularly in MCDA twins, that are difficult to identify with visual interpretation of the EEG. Significant correlation (ICC>0.6 with p <0.05) was found in all mathematical feature groups of the MCDA twins, and in most features — relative spectral power being the only exception. The frequency bands 3–8 and 8–15 Hz had more significant correlations compared to other frequency bands for the MCDA twins; while the early (72-hour postnatal age) time-point had more significant correlations compared to the other two time-points. Significant but fewer (two in total) correlations were evident in the DCDA twins, thus implying that hereditability of EEG characteristics in DCDA twins is not as strong as MCDA twins. These differences suggest that the significant ICCs in table 6-5 for all twins is likely due to the influence of EEG concordance of MCDA twins, with little to no contribution from the DCDA twins. With no obvious visual correlation between the EEG twin pairs, and without a formal structure to assess this correlation we had no means to quantify this similarity. This study therefore supports the demand for mathematical algorithms to compliment continuous EEG recordings in the NICU by providing strong results that are difficult to identify through visual interpretation. These algorithms could be of prognostic value in the near future (443).

Many features of preterm EEG are dependent on GA, therefore adjusting for age was important (439). Adjusting for age in the early time-point accounted for the varied GAs between the pairs, while adjusting for age at 32 and 35 weeks accounted for any potential difference in ex-utero maturation. Adjusting for age decreased the ICCs at the early time-point but not at 32 and 35 weeks, suggesting that the ICCs were affected more by the variability of the GA in the early time-point. Differences between intra- and extra-uterine development may not be as influential on EEG features as differences in GA, as demonstrated by previous work (444, 445).

In the MCDA twins, features within frequency bands 2 and 3 (3 – 8 Hz and 8 – 15Hz) had the most almost perfect ICCs (13/30) compared to 3/30 for the other two frequency bands. Artefact such as respiration occurs in the delta (0.5 – 3 Hz) frequency range and could be a reason for the lack of correlation in this band. Lack of delta frequency correlation was also evident in a recent study that examined ICC delta power in monozygotic (MZ) and dizygotic (DZ) twins during maturation over the first 3 months of life. Although MZ twins showed higher correlations in the higher delta frequencies (2.25 – 3.75 Hz), these results were not statistically significant (446). As previously reported in twin studies of older children, weaker correlation for this band is unsurprising as many physiological artefacts such as sweat sway and eye-movement occur at this frequency and could distort estimation of the features (430, 431). Our rudimentary artefact removal procedure, applied when estimating the features, is unlikely to eliminate these more subtle artefacts (436). Furthermore, factors such as epileptiform activity and environmental effects can influence delta waveforms. Common ictal electrographic epileptiform patterns and post ictal patterns are most frequently within the delta frequency range (329). Additionally, a study by Weeke et al. discovered a significant relation between the location of slower EEG and the infant's head position, strongly suggesting that slower patterns could be head position artefacts (328).

Symmetry analysis, as measured by inter-hemispheric coherence, was strong at both the early and 35-week time-point, however the inter-hemispheric coherence ICC values at 32 weeks were zero suggesting that physiological changes had a large impact on the inter-hemispheric coherence between 32 and 35 weeks. During this period, we know that the brain is developing rapidly with the disappearance of the subplate zone at 34 weeks and decreased contralateral thalamo-cortical projections (447). Genetically-linked neuronal migration and synaptogenesis occurs during this period (448) which could influence similar EEG patterns in MCDA twins (187). The early time-point provided the largest number of statistically significant ICCs, especially in the discontinuity category, however this decreased by 32 and 35 weeks. This could be due to the influence of the environment, which is known to impact neuronal connectivity (449). Another study reported how exposure to stressful factors such as intubation, is associated with decreased brain width in the frontal and parietal lobes, altered functional connectivity in the temporal lobe and altered diffusion measures of the brain (234). We did not see significant ICCs for the temporal organisation feature category at 35 weeks, however this was to be expected, with the EEG becoming more continuous by 34 weeks and IBI decreasing in duration.

No significant correlations >0.6 were seen in the age-matched singleton pairs, and limited amount of significant correlations were seen in the DCDA twins, while all four feature categories within the MCDA twins showed numerous, significant correlations. This supports the understanding that genetics and identical twining is a significant factor for background EEG (428, 429, 434, 450).

Visual analysis showed strong (>0.5) correlations within the twin pairs at all time-points, however we also found strong correlations in the age-matched singleton pairs. We found no difference between the within twins-pair correlations and within-singletons pair correlations at all time-points. Strong correlations were also evident between the MCDA and DCDA twin types, with no difference between the early, 32- and 35-week time-points (p =1.00, 1.00 and 0.874, respectively). This suggests that these strong correlations might be related to age similarity rather than the actual twining, with presence of preterm EEG features dependent on GA (175). Visual interpretation can identify short duration, transient or subtle waveforms, which cannot be identified in quantitative analysis such as abnormal delta brushes. We accept that the EEG assessment scheme may not be sensitive enough for twin comparison in preterm infants. As the assessment scheme was originally designed for grading abnormality, it may not be sensitive enough to uncover subtle characteristic differences within normal EEGs. Normal EEGs might influence the resulting comparisons, where the lower correlation could be influenced by the proportion of normal-to-abnormal features in the assessment scheme. Although the system might not be completely suited to this particular study, it does consider all known preterm EEG features.

We found that within twin EEG similarities could be disrupted by structural brain abnormalities; as deformed waveforms occurred in 71% of the EEG epochs where an IVH grade one or two was present. Our results showed that three of the four lowest correlations during the early time-point were from three twin pairs where either one or both infants had an IVH. A previous study reported that disorganised and dysmature patterns of the EEG can occur in infants with IVH, at a reported rate of 13% and 39% respectively (280). The abnormal waves feature sub-category (as illustrate in Figure 5-2, page 170) showed the most variability within the twin pairs, with deformed waves a prominent feature that differed especially when any CRUS brain abnormality was evident. By the 35-week time-point, the only infants with evidence of deformed waveforms were infants with evidence of IVH.

Early brain development is directly and heavily influenced by genetic factors. Early brain function and development such as neuronal connectivity, neural migration, synaptogenesis and apoptosis are dependent on specific epigenetic gene regulation, through DNA methylation and histone modifications (225). These genetic influences lead to unique neuronal connectivity and brain function developments, which will consequently differ between infants (228). A major site of neuronal connectivity and synaptic interaction is the subplate zone, where thalamo-cortical and cortico-cortical connections are processed. The development of thalamo-cortical and cortico-cortical connections strongly influence the EEG activity that can be recorded from the cerebral cortex (187, 447). As brain structures mature, the EEG will consequently develop and mature. Therefore, genetic factors indirectly influence the EEG due to its role in brain development. MCDA twins share all genetic interactions between alleles within and across genes, while non-identical twins share less than half (449), suggesting that neuronal connectivity in identical twins would be similar.

This is the first study to examine within-twin pair and between-twin group differences in the EEGs of preterm twins < 32 weeks GA, at three different time-points within the postnatal period. Consequently, it is difficult to directly compare our results to previous studies. One study by Vucinovic et al. examined sleep EEGs from 60 healthy, near-term twins and reported higher values of ICCs for EEG power in MCDA twins compared to DCDA twins in the first 3 months of life (434). No statistical difference was evident between the two types of twin groups in the absolute difference of EEG spectral power, however higher ICCs were evident in the alpha and beta frequencies in the MCDA twins. The delta frequency was recognised as the greatest mean absolute difference within the twin pairs and twin groups (434). In our study, we have shown most substantial correlations in the frequencies ranging from 3 – 15 Hz with the smallest correlations in the delta frequency range. We studied a different population of infants, at much earlier time-points, generated a comprehensive set of mathematical features to compliment spectral power analysis. Another key difference between the studies was that we investigated chorionicity/amnionicity, while Vucinovic et al. investigated zygosity. Although zygosity truly reflects genetic identity, we were not able to determine zygosity in our investigation due to the inability to explore DNA analysis of fetal membranes and placenta. Blood types, HLA typing, and the examinations of the placentas after delivery have been used to determine twin zygosity (451), however this information cannot be collected at the early time-points that we explored during this study, therefore chorionicity was the best alternative marker of zygosity.

It was difficult to control for influences such as infant handling, however during pruning of the early EEG, we selected only segments of good quality EEG with limited artefact. Sleep staging was not controlled for as our aim was to identify time-locked and synchronous EEG similarities within the twin pairs, ensuring that the twin pairs were in the same sleep state was difficult. Different sleep states could affect analysis, however as we used two-hour long epochs, it allowed enough time to capture state change in each epoch. During visual analysis, sleep states were considered in the cyclicity category in the temporal organisation sub-category of the assessment criteria (see Figure 5-2, page 170).

This study adds to our understanding of EEG in a high-risk population. Preterm brain injury is a significant problem, especially in preterm twins, it is therefore important to improve our understanding of brain development to improve our understanding of preterm twins during this vulnerable period. The main limitation is sample size. Twins represent a small proportion of the preterm population and excluding infants with IVH III/IV, cPVL and IUGR reduced this sample size further. Thus, collecting 10 preterm twin pairs <32 weeks represents recruitment over a 13-month period in a tertiary hospital, with 103 preterm infants admitted to the NICU. We missed a twin pair recording at 32 weeks as one twin was too unstable for a recording, in addition to two twin pair recordings and six singleton infant pairings at 35 weeks, due to early discharge. However, we managed to include 36 infants with normal CRUS findings into this study. To ensure the validity of our methods we additionally implemented a surrogate-data approach to eliminate non-significant ICC values for this sample size. A multicentre study could be a future solution for the small population, however this was beyond the scope for this research. The main strength of this study is that we comprehensively analysed the EEG with mathematical methods. In addition, we used long duration, multichannel video- EEG monitoring within the early postnatal period and repeat recordings to continuously monitor cerebral function in twin preterm infants. Specifically, for the MCDA and DCDA comparison, the twin pairs had synchronous EEG monitoring, to understand if any similarities were evident within twin pairs, ensuring no diurnal differences and that the twins were the same age. Furthermore, this is the first study, to our knowledge, that compares twin EEG monitoring in this vulnerable population at three different periods of the NICU stay, assessing the extent of maturation similarities.

In conclusion, this is the first study to report the influence of twinning on the preterm EEG and highlights the added value of mathematical analysis. The EEGs of MCDA twins show many more similarities than DCDA twins, suggesting a strong genetic influence during this early stage of development. Preterm brain injury remains a significant problem, especially in preterm twins, it is therefore important to improve our understanding of preterm brain development during the early postnatal period.

Chapter 7. Can EEG accurately predict 2-year neurodevelopmental outcome for preterm infants?

Part published as:

"Can EEG accurately predict 2-year neurodevelopmental outcome for preterm infants?"

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7.1. Introduction

Although the survival rate for preterm infants has improved in recent decades, they still continue to be at high risk of neurodevelopmental delay (452). Specifically, preterm infants <32 weeks gestational age (GA) are at an increased risk of cerebral palsy (CP) and other problems compared to more mature infants (423, 453). The prevalence of CP increases with decreasing GA: 6.9% at 24 - 26 GA, 4.2% at 27 – 31 GA and 1.0% at 32 – 34 GA (452). Social and emotional disorders such as depression, autism and attention deficit hyperactivity disorder are also well described (454-456), with preterm infants displaying more disorganised behaviours compared to full-term infants (457, 458).

Accurate recognition of infants at increased risk of abnormal neurodevelopment is challenging during the early neonatal period. By providing additional physiological information, like cerebral function, infants may benefit from early intervention, influencing positive outcomes (459). Immaturity at birth means that very preterm infants will stay in the NICU until they have achieved several milestones, including appropriate weight gain, temperature stability and until they have outgrown apneas and bradycardias. This means that infants may spend many months in the NICU before being discharged home. Within this period, their condition and development in the NICU can be monitored, but providing parents with an accurate prediction of outcome is challenging.

Continuous EEG monitoring is used to evaluate cerebral function (174, 175, 194, 409, 460) including the detection of seizures (321, 379, 395, 461). Certain characteristics of the EEG are more predictive of either a normal or abnormal outcome (194, 277). Previous studies have shown that early grading of the EEG (293, 307, 309, 321) and aEEG can predict long-term neurodevelopmental outcome (266, 303, 314-316, 341, 462). Hayashi-Kuriashi et al. analysed serials EEGs from preterm infants below 33 weeks GA at < day 6, day 7 – 19 and day 20 – 36, to predict outcome at 12/18 months (293). Results showed how abnormal activity from serial recordings in the first month of life predicted adverse outcome (293). Perivier et al. used serial EEG to predict 2-year outcome during the first week after birth (309). The EEG showed good specificity (95%), but poor sensitivity (16%) for the prediction of 2-year neurodevelopmental outcome.

In this current study, we used a carefully described and detailed assessment scheme to analyse the EEG at three different time-points during the infants' stay in the NICU, starting soon after birth. In contrast to previous studies, the initial recording commenced on day one of life and continued over the first 3 postnatal days, while other studies recorded at any time during the first week of life. In this study, we selected hourly time-points for analysis to ensure a comparable postnatal age. This study aims to establish whether serial multichannel video- EEG in the very preterm infant (<32 weeks GA), beginning on the first postnatal day, has a role in the prediction of outcome at 2 years of age.

7.2. Methods

7.2.1. Participants

This was a prospective, observational study performed between March 2013 – April 2014 in the NICU of Cork University Maternity Hospital. As seen in Figure 2-1 in chapter 2, all infants were from cohort 2 and were <32 weeks GA. Infants with known congenital anomalies were excluded. Infants were included in the study if they had early, continuous, multichannel EEG monitoring. Ethical approval was granted by the Clinical Research Ethics Committee of the Cork Teaching Hospitals, Ireland. Written informed parental consent was obtained.

The following clinical data was collected: GA, BW, Apgar score at 1 and 5 min, CRIB II score, initial pH, gender, major morbidities during neonatal course (such as IVH, PVL, sepsis, NEC, CLD and ROP), and medication during neonatal course.

7.2.2. Demographic and clinical data

Demographics and clinical details for all infants were collected from the medical notes and the Badger electronic database discharge summary document. Information regarding medication use was also obtained. AEDs were administered at the clinicians' discretion. The timing of administration was recorded, along with the administrations of other drugs such as morphine (120) and surfactant.

7.2.3. EEG Recording

Three EEG machines were used during the study period: the NicoletOne™ EEG system (CareFusion Co., San Diego, USA); the Nihon Kohden, EEG-1200, Neurofax, (Tokyo, Japan); and the Moberg ICU Solutions, CNS-200 EEG and Multimodal Monitor, (Ambler, Pennsylvania). EEG application was performed as soon as possible after birth when the infant was stable, following consultation with the clinical and nursing staff. Disposable Ambu Neuroline 700 Single Patient Surface Electrodes were applied to the scalp, using a modified neonatal version of the international 10/20 system (see chapter 2 pages 109-123 for further details).

EEGs were recorded at 3 time-points (EEG-1, EEG-32 and EEG-35) over the neonatal course, as previously described in chapter 2. EEG-1 was a continuous, long-term recording acquired as soon as possible after birth and continued until approximately 72 hours of age. Two-hour epochs of EEG were extracted at 4 different time-points (12, 24, 48 and 72 hours of age) to capture postnatal changes (463). Most recordings included all epochs, however situations such as late application of the EEG, instability of the infant, or poor EEG quality meant that some epochs were missing. Several periods during the early postnatal period were selected in order to capture EEG maturational changes occurring over the course of three days. The EEG-1 grade was based on the most abnormal grade selected from the 48-hour and 72-hour epochs. This was to account for situations when the EEG was initially abnormal, however improved by day 2 and 3 of age. Additionally, for comparison purposes, EEG-1 grade was also analysed based on the most abnormal grade from all 4 epochs. The whole recording was assessed for seizures, as previously described in chapter 4, and the background activity of the epochs were graded. EEG-32 and EEG-35 were shorter (2–4 hours) recordings at 32 weeks GA and 35 weeks GA, respectively. Table 7-1 illustrates the epochs that were visually analysed at each time-point.

EEG time-points	**Pruned 2-hour epochs for analysis**
EEG-1	12 hours, 24 hours, 48 hours, 72 hours
EEG-32	32 weeks GA
EEG-35	35 weeks GA

TABLE 7-1 TABLE ILLUSTRATING THE EPOCHS USED TO FOR EACH EEG PERIODS

7.2.4. EEG Grading

EEGs were graded based on a published standardised assessment scheme, described in chapter 5, which uses temporal organisation/cyclicity, normal features, abnormal waves and abnormal features for grading. This assessment scheme comprises five EEG grades. Grade 0 (normal); Grade 1 (mildly abnormal); Grade 2 (moderately abnormal); Grade 3 (severely abnormal); and Grade 4 (markedly abnormal). Abnormal grading groups are detailed in table 7-2.

Grade 1 **Mildly Abnormal**	**Grade 2** **Moderately Abnormal**	**Grade 3** **Severely Abnormal**	**Grade 4** **Markedly Abnormal**
Mildly Low Voltage	Moderately Low Voltage	Severely Low Voltage	-
Slightly prolonged Interburst Interval	Moderately prolonged Interburst Interval	Excessively prolonged Interburst Interval	-
Positive Temporal Sharps	Positive Rolandic Sharps	Seizures	Isoelectric
Sharps (Frontal, Central, Temporal and/or Occipital):	Deformed mechanical brushes	Burst Suppression (28wks <)	Status Epilepticus
	Deformed delta		
	Asymmetry		
	Abnormal Asynchrony		
	Brief Intermittent Rhythmic Discharges		
	Immature Waves (26wks <)		
	No cyclicity (28wks <)		

TABLE 7-2 EEG GRADING FOR PRETERM INFANTS CREATED FROM THE ASSESSMENT SCHEME IN CHAPTER 5. See page 170-176 for relevant definitions and user instructions for scheme.

For prediction of neurodevelopmental outcome, EEG grades were dichotomised to “normal EEG” (Grade 0-1) and “abnormal EEG” (Grade 2-4), as previously described (309, 464). In addition, the evolution of the EEGs over the course of the NICU stay (EEG-1 to EEG-35) was considered by categorising the EEGs as the following: remained normal; improved; deteriorated; or remained abnormal.

A neonatal electroencephalographer (RL[14]) graded all EEG time-points, blinded to all clinical information except for GA and administration of morphine/phenobarbitone. For validation,

[14] Rhodri Lloyd

another neonatal electroencephalographer (EP[15]) reviewed a random subset of 66 epochs from the recordings. Example of EEG grades are seen in figure 7-1.

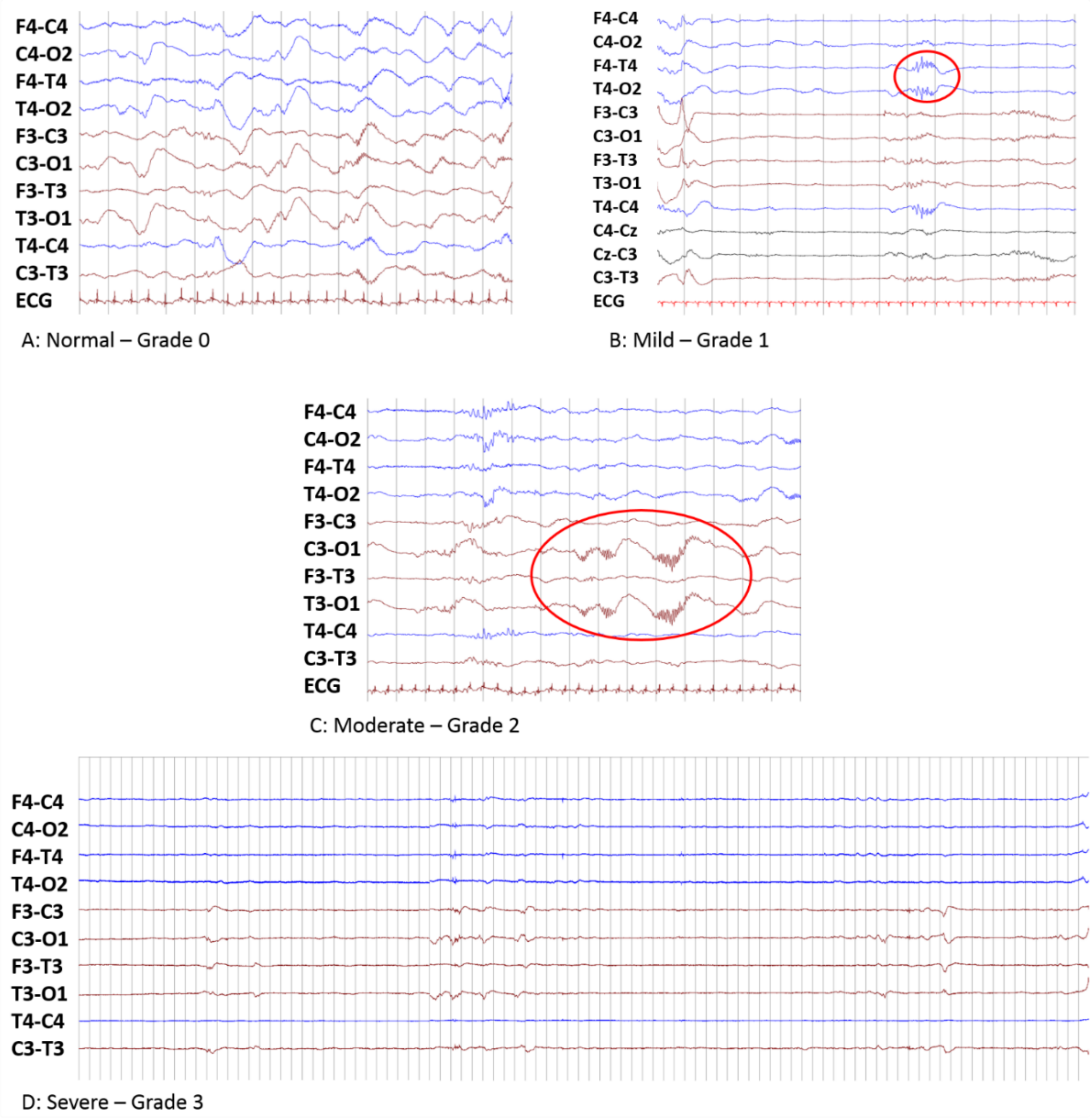

Figure 7-1 Examples of EEGs from four different infants, presenting varying degrees of EEG abnormal severity.

Infant A: Female, 30+3wGA at 12 hours of life, with continuous activity and no abnormal activity.

Infant B: Male, 30+2wGA at 12 hours of life, evidence of positive temporal sharps (PTS).

Infant C: Male, 26+0wGA at 12 hours of life, evidence of occipital deformed mechanical brush activity and asymmetry.

Infant D: Female, 26+4wGA at 48 hours of life, evidence of excessive discontinuity period lasting 95 seconds.

[15] Elena Pavlidis

7.2.5. Assessment of Neonatal Clinical Course

As described in chapter 3, five major clinical complications over the neonatal course (from admission to the NICU to discharge), were considered as high risk for later morbidity: grade 3/4 intraventricular haemorrhage (IVH)/ cystic periventricular leukomalacia (cPVL), chronic lung disease (CLD), necrotising enterocolitis (NEC), sepsis and retinopathy of prematurity (ROP), (see chapter 3, Table 3-1). Each infant was classified with either a 'complicated clinical-course score' based on the presence of at least one of these complications, or an 'uncomplicated clinical-course score' based on the absence of any of these complications (368-372). CRUS scan reports were reviewed for information regarding cerebral abnormality such as the grade of IVH or cPVL. Figure 7-2 illustrates an infant's course in the NICU through to neurodevelopmental follow up at 2 years of age.

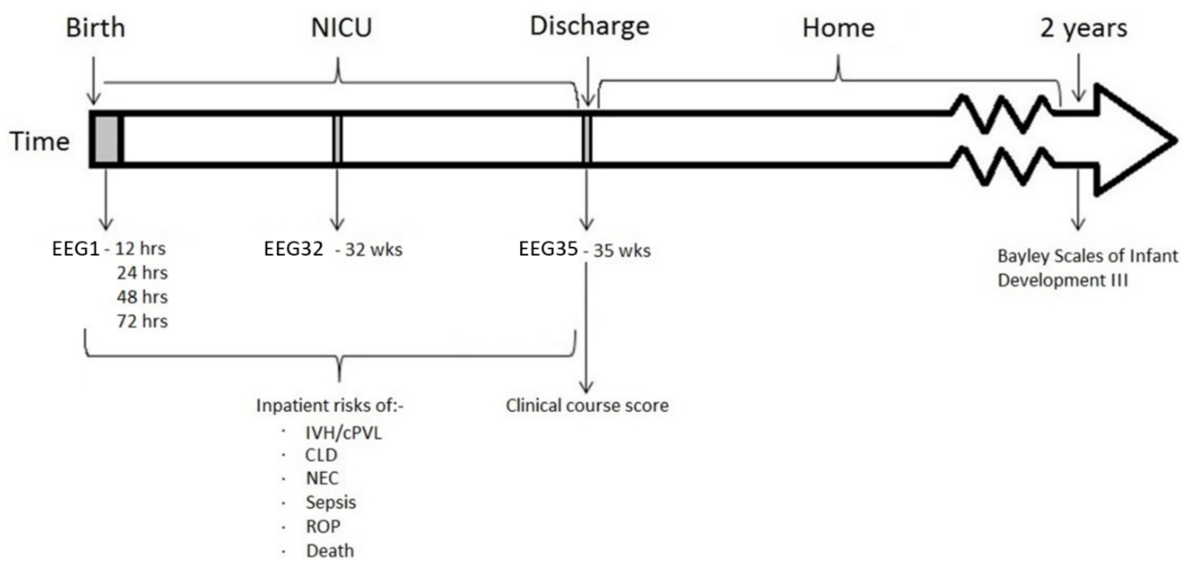

Figure 7-2 Timeline of infant's stay in the NICU through to the neurodevelopmental follow up at 2 years of age. This includes the timing of EEG recordings, period of possible complications, clinical course at discharge and Bayley-III score at 2 years of age. IVH, intraventricular hemorrhage; cPVL, cystic periventricular leukomalacia; CLD, chronic lung disease; NEC, necrotizing enterocolitis; ROP, retinopathy of prematurity

7.2.6. Two-year outcome assessment

The Bayley Scales of Infant Development III (Bayley-III) was used to assess neurodevelopmental outcome at 2 years corrected age. A research psychologist (EH[16]), a specialist neonatal physiotherapist (AMC[17]) and an occupational therapist (KN[18]) performed all assessments. The child's motor, cognitive and language development was assessed, as previously described in chapter 3. Abnormal outcome was defined as death, diagnosis of CP, or if any of the 3 subscales (motor, cognitive and language) were below one standard deviation from the mean; thus for the standardized scores, a value of less than 85 in any of the 3 subscales was deemed abnormal (310). A normal outcome, therefore, was identified when all 3 subscales were 85 or above.

7.2.7. Statistical Analysis

Inter-rater agreement between two raters was assessed using Cohen's kappa coefficient. Continuous variables were described using median and inter-quartile range (IQR) and categorical variables were described using numbers and percentages. For comparisons between the outcome groups (normal and abnormal), the Mann-Whitney U test was used for continuous variables and Fisher's exact test for categorical variables. Fisher's exact test was also used to assess the associations between outcome and EEG grading at each of the time-points, change in EEG grading over time and clinical course.

The AUC, sensitivity, specificity, PPV, and NPV and their corresponding 95% CIs quantified prediction of abnormal outcome for each EEG time-point. Additionally, these metrics were also used to test the association of clinical course and abnormal outcome, to test the association of combined EEG grade and clinical course with abnormal outcome. To test the independence of the EEG grades in predicting abnormal outcome and establish whether there were associations with any confounding factors, such as BW and other characteristics, the unadjusted and adjusted odds ratios (ORs) and their CIs were calculated from univariate and multivariate logistic regression analyses. Variables with $p<0.1$ in the univariate analysis were included in the multivariate logistic regression analysis giving adjusted OR and 95% CIs

[16] Emma Hennessy
[17] Anne-Marie Cronin
[18] Kannan Natchimuthu

for the association between EEG grade and outcome. Cochran's Q-test was used to investigate if EEG abnormality changed over the three time-points.
Statistical analyses were performed using MedCalc and IBM SPSS Statistics 21 (SPSS Inc, Chicago, Illinois). All tests were two-sided and a p-value <0.05 was considered statistically significant.

7.3. Results

During the study period, 103 infants <32 weeks GA were eligible and 70 were enrolled in the study. Thirty-three infants were not enrolled due to unavailability of EEG machines, consent decline, or lack of research staff availability. EEG recordings commenced within the first 72 hours of birth in all 70 infants. In total, 44 infants were singletons, 26 were twin individuals (13 twin pairs). Three infants with EEG recordings were excluded leaving 67 infants available for analysis and follow up (Figure 7-3). One twin pair were excluded from the study as one twin infant had a congenital anomaly. In addition, one infant was withdrawn from the study, following consent withdrawal.

At 2 years corrected age, 10 infants were lost to follow-up: 1 was excluded due to a late diagnosis of Beckwith–Wiedemann syndrome, 1 declined to attend appointments, and 8 did not respond. Outcome at 2 years, including infants that died, was therefore available in 57 of the 67 infants (85%). Four infants died prior to discharge from the NICU and 2 infants were diagnosed with CP by two years of age. The outcome of the remaining 51 infants were based on their Bayley III assessment. Forty (70%) infants had a normal neurodevelopmental outcome, and 17 (30%) had an abnormal outcome.

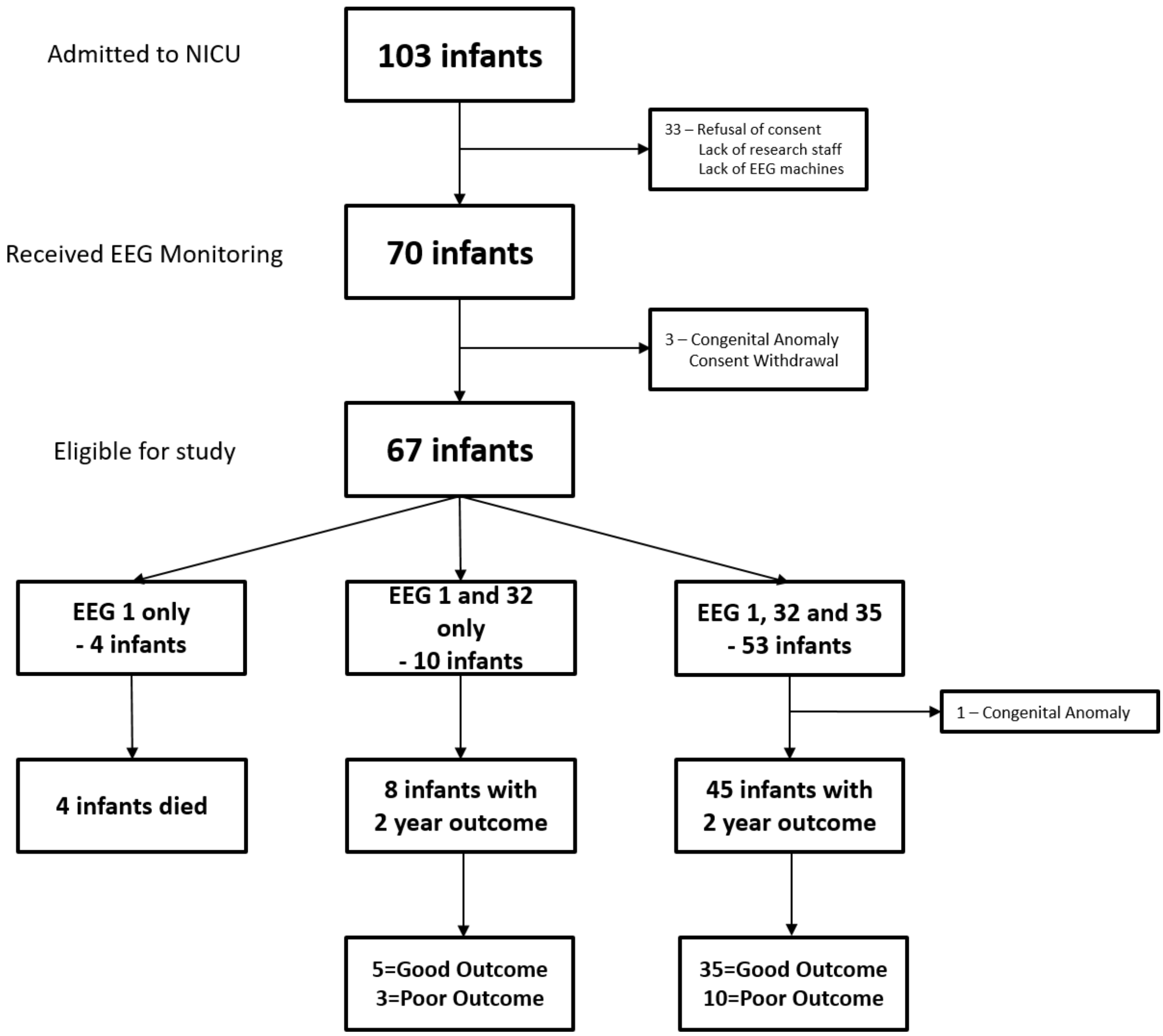

Figure 7-3 Flow chart showing number of infants recruited, with EEG recordings and neurodevelopmental follow up. EEG-1 refers to the recordings during the first 3 days of life, EEG-32 refers to the EEG at 32 weeks GA, and EEG-35 refers to the pre-discharge recording at 3 weeks GA

7.3.1. Demographic and clinical data

Clinical and demographic characteristics of the infants are described in table 7-3. GA ranged from 24+4 to 31+6 weeks, while BW ranged from 540g to 2250g. Phenobarbitone was the first-line anti-epileptic drug (AED) of choice, administered intravenously as a loading dose of 20 mg/kg.

	All infants (n=57) Median (IQR)
GA (weeks)	28.9 (26.5 – 30.4)
BW (g)	1160 (850 – 1445)
Apgar score 1 min	7 (5 – 8)
Apgar score 5 min	8 (7 – 9)
CRIB II	7 (4 – 10)
Initial pH	7.23 (7.19 – 7.29)
	n (%)
Gender (Male)	35 (61)
IUGR	8 (14)
CRUS/MRI brain abnormalities: -	
Normal	34 (60)
IVH Grade I/II	16 (28)
IVH Grade III/IV	6 (11)
cPVL	1 (2)
Complications: -	
Sepsis	27 (47)
Necrotizing Enterocolitis	10 (18)
Chronic Lung Disease	19 (33)
Retinopathy of Prematurity	2 (4)
Poor Clinical Course Score	36 (63)
EEG	
Abnormal during first 72 hrs (EEG1-)	39 (68)
Abnormal final EEG	24 (42)
Seizures	5 (9)
Medication	
AEDs	2 (4)
Morphine	14 (25)
Surfactant	11 (19)

TABLE 7-3 CLINICAL DEMOGRAPHICS AND CHARACTERISTICS OF ALL THE INFANTS AND OUTCOME. Key: IQR, interquartile range; GA, gestational age; BW, birth weight; g, grams; min, minutes; IUGR, intrauterine growth restriction; CRUS, cranial ultrasound; MRI, magnetic resonance imaging; IVH, intraventricular haemorrhage; cPVL, cystic periventricular leukomalacia; AEDs, anti-epileptic drugs; Abnormal final EEG, the individual's final recordings. In this instance 45 will be EEG-35 (infants that received all three recordings), in two instances it will be EEG-32 (infants that did not receive EEG-35 recording), and in 4 instances it will be EEG-1 (infants who only had one recording and died) (EEG-35, n=45; EEG-32, n=8; EEG-1, n=4).

7.3.2. EEG Recording

Data was available for 57 infants at EEG-1, 53 infants at EEG-32 (4 infants had died) and 45 infants at EEG-35 (8 infants were transferred, or discharged early). EEG-1 commenced at a median postnatal age of 7 hours 38 minutes (IQR: 4 hours 46 minutes – 11 hours 51 minutes). The median recording duration was 68 hours 24 minutes (IQR: 63 hours 57 minute – 71 hours 13 minutes). The median recording duration of EEG-32 and EEG-35 were 2 hours 46 minutes (IQR: 2 hours 1 minute – 4 hours 4 minutes) and 2 hours 11 minutes (IQR: 2 hours – 2 hours 59 minutes), respectively.

7.3.3. EEG Grading

Inter-rater agreement for grading of EEGs (Normal vs Abnormal) was found to be high (Cohen's kappa coefficient = 0.91).
For each infant, the grade of EEG at each time-point throughout the NICU stay is shown in table 7-4, while EEG grades across infants at each time-point in the study are illustrated in figure 7-4.

Infant	Complications Day 1	EEG 12 hrs	EEG 24 hrs	Complications Day 2	EEG 48 hrs	Complications Day 3	EEG 72 hrs	EEG-1	Complications 72hrs-32wks	EEG-32	Complications 32 - 35wks	EEG-35	Complications after 35wks	2 year Outcome
1		2	2		2	IVH4	x	2		2		2		0
2	Evolving CLD	2	2	Sepsis	2		2	2	D8 – IVH2 D9 – Sepsis	2		2		0
3		2	2		2	IVH2	2	2	D25 – Sepsis D26 – NEC	2		2		0
4		2	2	IVH4	3		2	3	D12 – Sepsis **D24 - RIP**	x		x		1
5	Evolving CLD	2	2		2		x	2	D9 – IVH1	2		x		0
6	Evolving CLD	0	0		2		1	2		1		1	D70 – Sepsis	0
7		2	2		2	IVH2	2	2	D37 - NEC D37 – Sepsis	1		1		0
8		x	0		0		1	1	D4 – IVH2	0		x		0
9		0	0		2		0	2	D8 – IVH1	0		x		0
10		1	1		2		2	2	D8 – IVH1	1		x		0
11	Evolving CLD	1	2		0		2	2	D12 – Sepsis	2	D43 - Sepsis	2		1
12		2	2		3	IVH3	2	3	D4 – Sepsis D22 – NEC **D24 – RIP**	x		x		1
13		1	1		1	Sepsis	1	1		1		1		0
14		2	2		2		2	2	D6 – IVH1	2		2		1
15	Evolving CLD	x	x	Sepsis	3	IVH2	2	3		2	D34 - Sepsis	2		1
16		x	2		2		2	2		2		x		1
17	Evolving CLD	0	0		2	Sepsis	0	2		0		0	D88 - NEC	0
18	Evolving CLD	2	2		2	IVH4	X	2		2		2		0
19		2	0		0		2	2		2		0		0
20		x	2		2		x	2		1		0		0
21		1	3		2		X	3	**D6 - RIP**	x		X		1
22		2	2		2	Sepsis	X	2		2		1		0
23		1	1		1		x	1	D10 - Sepsis	2		2		1
24		1	1		1	IVH1	1	1		1		X		0
25	Evolving CLD Sepsis	2	2		2	NEC	2	2	D19 - NEC	2		2		1
26	Evolving CLD	1	1		1	IVH1	X	1	D35 - Sepsis	1		1	ROP	0
27	Evolving CLD Sepsis	2	2		2	IVH4	2	2	**D31- RIP**	x		x		1
28	Evolving CLD IVH4 Sepsis	2	2		2		2	2	D32 - NEC	2		2		0
29		1	1		0		0	1		0		0		0
30	Evolving CLD Sepsis	1	1		0		x	1		1		1		0
31	Evolving CLD	2	2		2		x	2		2		2		1
32		2	2		2	Sepsis	2	2		2		2		1
33		0	2	Sepsis	2		X	2		0		0		0
34	Evolving CLD	1	1		1	Sepsis	1	1	D10 - Sepsis	1	D36 - NEC	0		0
35		1	0		0		0	1		1		1		0
36		1	1	Sepsis	1		1	1		1		1		0

37		1	0		1		1		**1**		**1**		**1**		0
38		1	1		1		1		**1**		**1**		**0**		0
39		2	3		2		2		**3**		**2**	D26 - cPVL	**x**		1
40		0	0	IVH2	0		0		**0**		**1**		**1**		0
41	Evolving CLD	0	0		0		0		**0**		**1**		**1**		0
42		0	0		0		0		**0**		**1**		**0**		0
43	Evolving CLD	2	2		2		2		**2**		**2**		**1**		0
44		2	2		2		2		**2**		**2**		**x**	ROP	1
45	Evolving CLD	2	2		2	IVH2	2		**2**		**2**		**1**		0
46		0	0		0		0		**0**		**0**		**0**		0
47		2	2		2	Sepsis	2		**2**		**2**		**2**		1
48		x	2		2		2		**2**	D9 – Sepsis	**0**		**0**		0
49		x	2		2		2		**2**		**1**		**1**		0
50		X	0		1		1		**1**	D6 – Sepsis	**1**		**0**		0
51		2	2		2	Sepsis	X		**2**	D11 - NEC	**2**		**2**		1
52		0	1		2	IVH1	1		**2**		**1**		**0**		0
53		1	1		2	IVH1	X		**2**		**2**		**1**		0
54		x	1		1		1		**1**		**1**		**1**		0
55	Evolving CLD Sepsis	2	2		2		X		**2**	D10 – IVH2 D26 - NEC	**2**		**2**		0
56	Evolving CLD	x	3	Sepsis	2		2		**3**	D13 - Sepsis	**2**		**2**		1
57		2	2		2		2		**2**		**2**		**0**		0

TABLE 7-4 ALL GRADES OF EACH EEG RECORDING IN ADDITION TO THE COMPLICATIONS THE INFANTS EXPERIENCED DURING THEIR HOSPITALIZATION. The early EEG scores are composite scores of the early epochs. Key: Complications, major complications of morbidity risk; IVH, intraventricular haemorrhage; cPVL, cystic periventricular leukomalacia; CLD, chronic lung disease; NEC, necrotizing enterocolitis; ROP, retinopathy of prematurity; D, day; 0 (EEG score), normal; 1 (EEG score), mildly abnormal; 2 (EEG score), moderately abnormal; 3 (EEG score), severely abnormal; x (EEG score), missing data; 0 (outcome score), normal; 1 (outcome score), abnormal.

In total, 18 infants (32%) had a normal EEG-1 (grade 0 and 1) and 39 (68%) had an abnormal EEG-1 (grade 2 and 3). No EEG epoch was graded isoelectric or status epilepticus (grade 4). The percentage of abnormal EEGs decreased between the three time-points (68% vs 49% v 36%, $p<0.001$, n=45). The most common type of abnormality from all time-points was deformed waveforms, which was evident in 86% of the abnormal epochs. By EEG-35, 29 infants (64%) had a normal EEG and 16 (36%) had an abnormal EEG, while again the deformed waveform was most common type of abnormality, evident in 75% of the abnormal epochs.

In total 23 infants had brain injury (any grade IVH or cPVL), of which 19 had an abnormal EEG-1 grade in the first 72 hours. The other four infants with normal EEG-1 all had IVH grade 1 or 2, with three abnormalities reported within 72 hours and one infant on day 4 of life. Of the 7 infants with IVH III/IV or cPVL, 88% (23/26) of epochs within the EEG-1 time-point showed evidence of deformed waveforms. All six infants with IVH III/IV, showed CRUS abnormality within 72 hours of life. Also evident were mechanical brushes, 58% (15/26); PRS, 50% (13/26); asymmetry 8% (2/26); asynchrony 34% (9/26); severe/moderate low voltage, 4% (1/26); and excessive/moderate discontinuity 23% (6/26). EEG-35 was recorded in 3 infants with IVH III/IV or cPVL, showing deformed waveforms and mechanical brushes in two cases, while the other infant showed asynchrony.

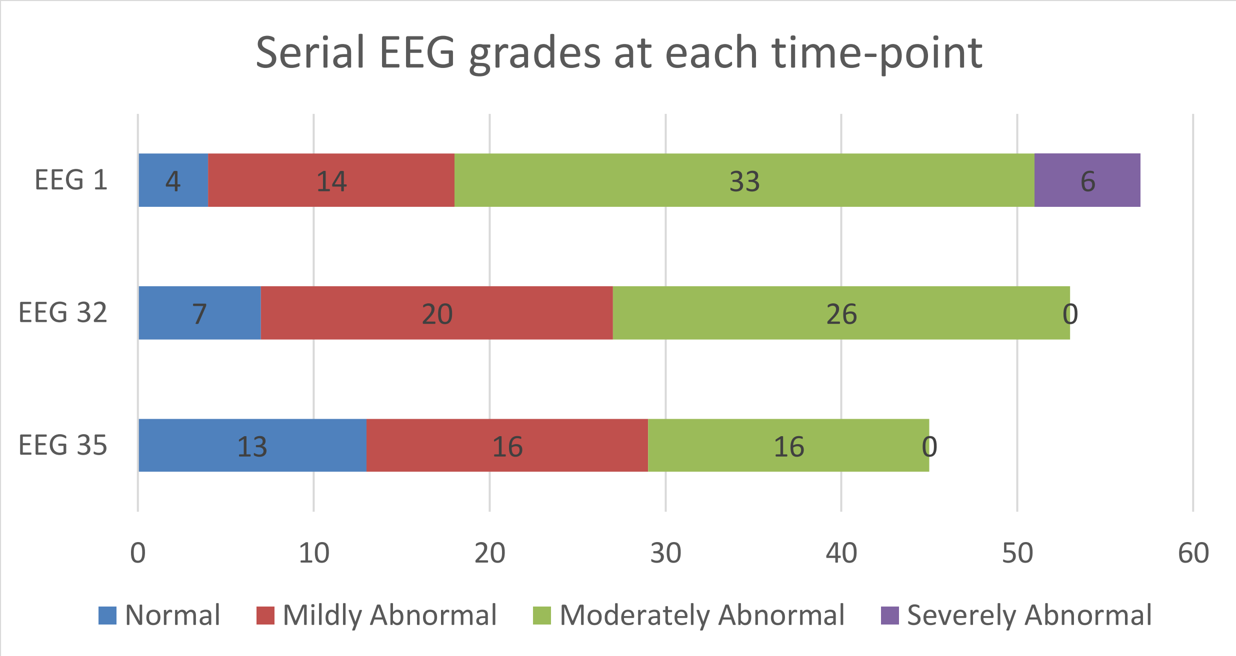

Figure 7-4 Serial EEG grading during monitoring.

7.3.4. Demographic characteristics and Outcome Assessment

Using the Bayley III assessment 40 (70%) infants had a normal neurodevelopmental outcome, and 17 (30%) had an abnormal outcome, including two with CP and 4 that died. Moderate-severe neurodevelopmental delay was evident in 4 infants; two infants with CP, and two other infants that scored at least one Bayley subscale below 70.

Clinical and demographic characteristics of the infants and their relationships with outcome are detailed in table 7-5. Only IUGR was associated with an abnormal outcome (29% (5/17) in abnormal group had IUGR versus 8% (3/40) in the normal group, p=0.043).

	Normal Outcome (n =40) Median (IQR)	Abnormal Outcome (n =17) Median (IQR)	p-value [a]
GA (weeks)	29.0 (26.6 – 30.5)	28.6 (26.4 – 29.9)	0.447
BW (g)	1185 (890 – 1563)	1140 (625 – 1335)	0.084
Apgar score 1 min	7 (5 – 8)	6 (4 – 8)	0.052
Apgar score 5 min	9 (7 – 9)	8 (7 – 9)	0.056
CRIB II	7 (3 – 10)	8 (4 – 12)	0.363
Initial pH	7.23 (7.18 – 7.28)	7.24 (7.18 – 7.34)	0.427
	n (%)	n (%)	p-value [b]
Gender (Male)	25 (63)	10 (59)	1
IUGR	3 (8)	5 (29)	0.043
CRUS/MRI brain abnormalities: -			
Normal	23 (58)	11 (65)	0.089
IVH Grade I/II	14 (35)	2 (12)	
IVH Grade III/IV	3 (8)	3 (18)	
cPVL	0 (0)	1 (6)	
Complications: -			
Sepsis	16 (40)	11 (65)	0.146
Necrotizing Enterocolitis	7 (18)	3 (18)	1
Chronic Lung Disease	13 (33)	6 (35)	1
Retinopathy of Prematurity	1 (3)	1 (6)	0.511
Medication			
AEDs	1 (3)	1 (6)	0.511
Morphine	8 (20)	6 (35)	0.314
Surfactant	9 (23)	2 (12)	0.476

TABLE 7-5 CLINICAL DEMOGRAPHICS AND CHARACTERISTICS OF ALL THE INFANTS AND COMPARING INFANTS WITH A GOOD AND POOR OUTCOME. Key: IQR, interquartile range; GA, gestational age; BW, birth weight; g, grams; min, minutes; IUGR, intrauterine growth restriction; CRUS, cranial ultrasound; MRI, magnetic resonance imaging; IVH, intraventricular haemorrhage; cPVL, cystic periventricular leukomalacia; AEDs, anti-epileptic drugs (first-line anti-epileptic drug (AED) of choice, administered intravenously as a loading dose of 20 mg/kg).

[a] Mann Whitney U-test

[b] Fisher's exact test

7.3.5. EEG and Outcome Assessment

All three serial EEGs were individually predictive of an abnormal outcome, when the subscales were combined (Table 7-6). AUC for EEG-1 was 0.68 (95% CI: 0.55 – 0.80), p=0.030; EEG-32 was 0.84 (95% CI: 0.70 – 0.92), p <0.001; and EEG-35 was 0.91 (95% CI: 0.83 – 1.00), p<0.001. For comparison, the AUC for the presence of IVH III/IV or cPVL was 0.58 (95% CI: 0.41 – 0.75), p=0.342. In this table, the sensitivity and specificity also increases with each time-point. For direct comparison purposes, table 7-6 also provides results when only the infants that had all serial EEG recordings were included.

		EEG	Outcome Normal	Abnormal	p-value[a]	AUC (95% CI)	Sens. (95% CI)	Spec. (95% CI)	PPV (95% CI)	NPV (95% CI)
EEG-1	**All infants (n = 57)**	Normal (n=18)	17	1	0.011	0.68 (0.55 – 0.80)	94 (71 – 100)	43 (27 – 59)	41 (26 – 58)	94 (73 – 100)
		Abnormal (n=39)	23	16						
	Infants with EEG at every time-point (n = 45)	Normal (n=16)	15	1	0.071	0.66 (0.51 – 0.80)	90 (56– 100)	43 (26 – 61)	31 (15 – 51)	94 (70 – 100)
		Abnormal (n=29)	20	9						
EEG-32	**All infants (n = 53)**	Normal (n=27)	27	0	<0.001	0.84 (0.70 – 0.92)	100 (75 – 100)	68 (51 – 81)	50 (30 – 70)	100 (87 – 100)
		Abnormal (n=26)	13	13						
	Infants with EEG at every time-point (n = 45)	Normal (n=23)	23	0	<0.001	0.83 (0.68 – 0.92)	100 (69 – 100)	66 (48 – 81)	46 (24 – 68)	100 (85- 100)
		Abnormal (n=22)	12	10						
EEG-35	**All infants (n = 45)**	**Normal (n=29)**	**29**	**0**	**<0.001**	**0.91 (0.83 – 100)**	**100 (69 – 100)**	**83 (66 – 93)**	**63 (35 – 85)**	**100 (88- 100)**
		Abnormal (n=16)	**6**	**10**						

TABLE 7-6 EEG DURING FIRST 72 HOURS, 32 WEEKS AND 35 WEEKS PREDICTING 2 YEAR OUTCOME IN ALL AVAILABLE INFANTS. Including a comparison between all the infants and only the infants (n=45) who had an EEG recording at all 3 time-points. All infants with an EEG-35 recording received EEG recordings at both the first and second time-points.

[a] *Fisher's exact test*

The clinical variables BW, Apgar at 1 and 5 minutes, IVH and IUGR were tested as possible confounding factors. Table 7-7 presents the unadjusted ORs for EEG-1 and also results when controlled for these possible confounding variables. The EEG-1 remained statistically significant in each of the multivariable logistic regression analyses (ORs range: 9.14 – 13.96), when the potential confounding variables were controlled for. None of the potential confounding variables were statistically significant. Multivariable logistic regression was not achievable for EEG-32 and 35 due to zero entries in the confusion matrix: no infant with a normal EEG at 32- or 35-weeks GA resulting in an abnormal neurodevelopmental outcome.

	Odds Ratio (95% CI)	p-value
Unadjusted		
EEG-1	**11.83 (1.43 – 98.06)**	**0.022**
Adjusted		
EEG-1	**9.65 (1.12 – 82.81)**	**0.039**
Weight	1.00 (1.00 – 1.00)	0.332
EEG-1	**9.75 (1.14 – 83.17)**	**0.037**
Apgar 1 min	0.87 (0.68 – 1.12)	0.276
EEG-1	**9.14 (1.07 – 78.03)**	**0.043**
Apgar 5 min	0.67 (0.43 – 1.04)	0.075
EEG-1	**10.51 (1.24 – 89.17)**	**0.031**
IUGR (Ref: Normal)	4.14 (0.77 – 22.15)	0.097
EEG-1	**13.96 (1.56 -124.64)**	**0.018**
CRUS/MRI (Ref: Normal)		0.126
IVH Grade 1/2	0.19 (0.03 - 1.08)	
IVH Grade 3/4 or cPVL	1.32 (0.23 – 7.46)	

TABLE 7-7 RESULTS OF MULTIVARIATE LOGISTIC REGRESSION TESTS WITH ONLY THE POSSIBLE CONFOUNDING CLINICAL VARIABLES TESTING THE ASSOCIATION WITH THE EEG-1 RECORDING.

When analysing the EEG over time from EEG-1 to EEG-35, the EEG has the potential to remain normal, remain abnormal, improve or deteriorate. Table 7-8 shows the trajectory of the grades at different time-points and that maturation of the EEG grades over time may be useful for the prediction of outcome ($p < 0.001$).

	Outcome (n=57 – All infants)			Outcome (n=45 - Infants with EEG at every time-point)		
EEG Maturation	**Abnormal n (%)**	**Normal n (%)**	**p-value [a]**	**Abnormal n (%)**	**Normal n (%)**	**p-value [a]**
EEG Always Normal	0 (0)	17 (100)	<0.001	0 (0)	15 (100)	<0.001
EEG Normalised	0 (0)	17 (100)		0 (0)	14 (100)	
EEG Deteriorated	1 (100)	0 (0)		1 (100)	0 (0)	
EEG Always Abnormal	16 (73)	6 (27)		9 (60)	6 (40)	

TABLE 7-8 NUMBER OF PRETERM INFANTS WITH DIFFERENT EEG GRADE EVOLUTIONS FROM EEG-1 TO EEG-35 AND THEIR NEURODEVELOPMENTAL OUTCOME

The EEG considered for this analysis was the first and the last recording for each infants.

EEG Always Normal – when EEG-1, EEG-32 and EEG-35 were normal;

EEG Normalised – when EEG-1 was abnormal, and EEG-35 was normal;

EEG Deteriorated – when EEG-1 was normal, and EEG-35 was abnormal;

EEG Always Abnormal – when EEG-1, EEG-32 and EEG-35 were abnormal.

[a] *Fisher's exact test*

Forty-four percent (17/39) of the infants with an abnormal EEG-1 normalised by the pre-discharge recording. All infants whose EEG remained normal or was initially abnormal but normalised over the course of the three recordings had a normal 2-year outcome.

One infant with abnormal outcome had a normal EEG-1, then became abnormal by EEG-32 following an episode of sepsis. When the EEG was initially abnormal and remained abnormal from EEG-1 to EEG-35, 60% had an abnormal outcome. Six infants had a normal 2-year outcome even though the EEGs were abnormal at all time-points. All had major complications within the first 72 hours. All of these infants had an IVH II-IV (CRUS recording ranging from day 1 – 10), 4 had an episode of sepsis, 4 developed CLD, and 3 had an episode of NEC. In these instances, the four time-points within the EEG-1 were inspected, which showed that every time-point in every infant was abnormal, even at 12 hours. There were deformed waveforms (Figure 7-5) in all four time-points of EEG-1, except at a 12-hour time-point in two infants. One infant instead demonstrated mechanical brushes, while the other demonstrated PRS waves. At EEG-32 and 35 recordings, deformed waveforms were evident in all but one infant, who alternatively demonstrated frequent asynchrony.

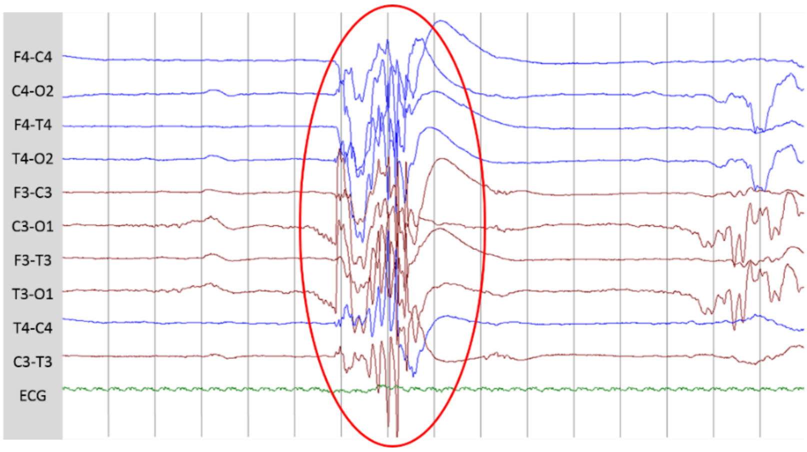

Figure 7-5 EEG example of deformed waveforms from a male, 30+0 weeks GA infant at 48 hours of age.

In the 16 infants whose EEG stayed abnormal and who had an abnormal outcome, due to missing data (eleven), 37 EEG time-points were analysed. Deformed waveforms and/or mechanical brushes occurred in all, except for one time-point. Other abnormal features, however, such as lack of cyclicity or asymmetry, appeared in conjunction deformed and/or mechanical brushes. IVH (I-IV) and cPVL was evident in 6 of the infants, 10 had sepsis, 6 developed CLD, 3 had NEC and 1 developed ROP.

Of the four infants that died during monitoring three had abnormal EEG grades from the outset while one 24-week infant with IUGR initially had a normal EEG at 12 hours, but this became severely abnormal by 24 hours. Two infants had seizures during the early postnatal period. Additionally, to the infants that died, two infants from the cohort were diagnosed with CP at two years of age, one with evidence of cPVL. This infant had a moderately abnormal EEG from the 12-hour epoch to the final EEG-32 recording, showing very suppressed amplitudes and excessive discontinuity. PRS were seen in the recordings at the 72 hour epoch and was also evident at EEG-32. The second infant with confirmed CP had a moderately abnormal grade at every recording, which included periods of deformed waves, moderately low voltage, moderately prolonged IBI for age (20 – 50 seconds), and asymmetry. In total 14 infants showed evidence of PRS during the early postnatal period, of which 1 had cPVL, 4 had IVH grade 3/4 and 3 had IVH grade 1/2. Three of these infants also showed the activity at EEG 32 and 35; two of these infants had a very poor clinical course with three major complications each during stay in the NICU, while the other infant had cPVL.

7.3.6. Clinical Course and Outcome Assessment

Table 7-9 shows the diagnostic accuracy (AUC, sensitivity, specificity, PPV and NPV) of the clinical course to predicting neurodevelopmental outcome at 2 years. When only the 45 infants with all EEG time-points were analysed, an AUC of 0.65 (0.47-83), sensitivity of 90% (56-100%) and specificity of 40% (24 – 58%) was reported.

	Normal Outcome	Abnormal Outcome	p-value [a]	AUC (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Uncomplicated Clinical Course (n=15)	14	1	0.077	0.65 (0.47 – 0.83)	90 (56 – 100)	40 (24 – 58)	30 (23 – 38)	93 (68 – 99)
Complicated Clinical Course (n=30)	21	9						

TABLE 7-9 UNCOMPLICATED AND COMPLICATED CLINICAL COURSE PREDICTING 2-YEAR OUTCOME IN ALL AVAILABLE INFANTS WITH ALL EEG TIME-POINTS, n=45.

[a] *Fisher's exact test*

Table 7-10 shows the diagnostic accuracy of combining clinical-course score and EEG-35 (n=45) for predicting outcome. Of the 10 infants with abnormal outcome, 9 infants had an abnormal EEG-35 and poor clinical course, while one infant had an abnormal EEG-35 but a good clinical course. Of the 35 infants with normal outcome, 14 infants had a normal EEG-35 and good clinical course, while 15 infants had a normal EEG-35 but a poor clinical course. Furthermore, no infants with a normal outcome had a good clinical course but an abnormal EEG-35.

	EEG-35	Normal Outcome	Abnormal Outcome
Uncomplicated Clinical Course (n=15)	Normal (n=14)	14	0
	Abnormal (n=1)	0	1
Complicated Clinical Course (n=30)	Normal (n=15)	15	0
	Abnormal (n=15)	6	9

TABLE 7-10 COMBINATION OF CLINICAL COURSE AND EEG-35, PREDICTING 2-YEAR OUTCOME IN ALL AVAILABLE INFANTS n=45.

7.4. Discussion

We report the utility of serial multichannel EEG recordings in preterm infants for the prediction of neurodevelopmental outcome at 2 years of age. EEGs were recorded in very preterm infants during the first 3 postnatal days and at approximately 32 and 35 weeks. All recordings at each time-point proved useful, however EEG-35 had the highest AUC of 0.91 [95% CI: 0.83 – 100]; Sensitivity = 100 [95% CI: 69 – 100]; Specificity = 83 [95% CI: 66 – 93]). At this time-point, infants are less vulnerable to complications and more stable. Infants with normal or mildly abnormal EEG recordings at approximately 35 weeks GA had normal outcomes in every case. This finding suggests that an EEG at 35-weeks could offer valuable prognostic information for healthcare teams and parents.

The high predictive performance of the EEG could be a result of the newly proposed assessment scheme used. It is a very detailed age-specific scheme which has numerous features incorporated. The four EEG categories include; temporal organisation, normal waves, abnormal waves and abnormal features. Visual EEG interpretation is challenging as features change with maturation (411, 412) therefore using an age-specific preterm assessment scheme can enhance a more standardised way of analysis. The strong inter-rater agreement results (Cohen's kappa of 0.91) confirm this.

Recent studies have used serial EEG abnormality to predict outcome in very preterm infants (293, 307, 309), identifying later recordings as more useful. In these studies, adverse outcome was associated with infants with more severe EEG abnormalities, such as seizures, acute and chronic stage abnormalities, or abnormal features. Sensitivity and specificity to predict outcome ranged from 16 – 83% and 88 – 96% respectively (293, 307, 309), in comparison to our results of 100% and 83%. Our results are not completely comparable however, due to numerous methodological differences between the studies, such as different EEG grading scheme, a different recording duration such as 45 min – 1hr (293, 307, 309); and the serial EEGs performed depended on the infants' chronological age (293), or the last recording at different age such as 33 weeks GA (309). Studies have also reported an association between aEEG/limited EEG and abnormal outcome, with sensitivity and specificity values ranging from 73 – 93% and 41 – 97%, respectively (315, 341, 462). Particular features such as early depression, absent cyclicity, seizures, prolonged IBI, burst suppression and specific characteristics of burst activity were predictive of an abnormal outcome (303, 314-316).

This study has shown different findings to our previous study in chapter 3, which used the Watanabe EEG grading system in a model with other physiological measurements, with a different group of infants. We reported a sensitivity of 50%, a specificity of 89% and AUC value of 0.69 for the EEGs prediction of poor outcome at 2 years of age within the first 24 hours of life (464). This earlier study used early recordings only i.e. equivalent to the EEG-1 time period from this current study, which showed similar AUC (0.68), however the sensitivity was higher at 94% while the specificity was lower at 43%. This could be due to study differences, such as; different infants in both cohorts, different grading system used, different epochs used (with the current study also using 48- and 72-hours epochs), and also that 2 hour epochs were used for this current study while 1 hour was used in the previous. The latter meant that the current study had twice the amount of data available for analysis, increasing the ability to identify more abnormalities, which could be a reason why abnormalities were seen in 68% of the EEG-1 time-point in the current study and only 26% in the pervious. Additionally, over a two-hour period, cyclicity and staging is more identifiable, allowing for more accurate analysis. This may explain why the EEG-1 decreased in specificity and increased in sensitivity, compared to the previous study.

Even though EEG-1 provided low specificity, PPV, and the lowest AUC, the percentage of infants with an abnormal outcome was significantly higher in the group with an abnormal EEG-1 compared with the group with a normal EEG-1. This difference remained after adjusting for the potential confounding effects of weight, Apgar score, IUGR and IVH grade. In addition, EEG-1 was superior to the clinical characteristics in the prediction of outcome. This was also found in a previous study by Hayashi – Kurahashi et al. reporting that risk factors such as small for GA, CLD, postnatal corticosteroids and brain injury were inferior to the EEG for the prediction of outcome (293). This study also recorded the EEG at three periods, and reported how the specificity increased with time when predicting developmental delay and CP at 12 to 18 months of age, however the sensitivity decreased (293). Contrastingly, to our results, both sensitivity and specificity increased over time.

We have found that a normal EEG at 32 and 35-weeks predicted normal outcome at 2-years in our group of infants. At 35 weeks, both sensitivity and specificity were highest (100% and 83%, respectively), with PPV and NPV of 63% and 100% respectively. These PPV results were higher (63%) compared to CRUS and MRI studies at term-equivalent age: 46 – 61% (465) and 27 – 32% (465, 466) respectively. The suitability of pre-discharge CRUS and MRI recordings has previously been discussed (467), however this study offers a potential role for EEG at pre-discharge (approx. 35 weeks). Additionally, the EEG-1 recording was a better predictor of abnormal outcome than CRUS, with an AUC of 0.68 (0.55 – 0.80, p=0.011) and 0.58 (0.41 – 0.75, p=0.342) respectively, suggesting that early EEG assessment of background activity have a role to play in the early postnatal period, with previous reports suggesting that abnormal EEG findings can precede abnormalities in CRUS (316, 468).

Background EEG activity evolves gradually with maturity in preterm infants, therefore serial recordings can identify deteriorating or improving brain function following a resolving acute injury (307, 469). We report that 44% of the infants with an abnormal EEG-1 improved by the 35-week recording, and all infants with normal 35-week EEG regardless of prior EEG findings, had a normal 2-year outcome. Furthermore, as standard, the clinician would be aware of a complicated clinical course in infants. Adding 35-week EEG to the clinical course score could compliment current clinical practice. This is especially beneficial for infants with a poor clinical course as a normal EEG prior to discharge is a positive prognostic indicator.

One infant had a normal EEG-1 but then deteriorated later due to sepsis and the final two EEGs were abnormal. Sepsis is known to increase vulnerability of the brain due to inflammation and white matter damage (367); this infant had an abnormal neurological outcome (371). We cannot definitively say that sepsis was the cause of EEG deterioration, especially as other infants with normal recordings and normal outcomes also suffered sepsis, however in this particular case the EEG displayed a general deterioration in background activity. The infant did not have CRUS or MRI abnormalities, reflecting the ability of the EEG to document function changes in brain activity (54, 276, 400). In this study, all 7 infants with IVH grade III/IV or cPVL, had abnormal background EEG patterns during the early postnatal period, which did not resolve following injury. The brain is at highest risk of injury during the early postnatal period, due to the fragile capillary network in the preterm brain and the reduced autoregulatory control (48).

Deformed delta waves and mechanical brushes were prominent in infants with abnormal EEG patterns, however they appeared intermittently in both infants with normal and abnormal outcomes. However, in infants with abnormal outcomes, these waveforms occurred in association with other abnormal features such as PRS or increased discontinuity. Deformed waves have previously been described as a disorganised pattern of a chronic stage abnormality and are believed to provide valuable information regarding outcome (470). Disorganized patterns are believed to be associated with acute brain injury and poor outcome (194, 280, 281, 307). In our cohort, the presence of deformed waveforms in infants who had normal neurodevelopmental outcome suggests that these waveforms are seen in response to an acute event that may represent an important biomarker of abnormal brain function. If the clinical condition resolves, these waveforms can disappear. Two review studies report that PRS is a marker for specific brain injury/PVL (277, 312), however this was not clearly evident in our study. We report the occurrence of PRS in 13 infants, 8 of which had a grade of IVH (grade I – IV) or cPVL, two of which developing CP. Only three infants showed PRS in the EEG-32 and 35 recordings, one of which developed CP. This may suggest that persistence of PRS over a long period of time could reflect in a more adverse outcome, however minimal, sporadic appearances may be less concerning. Future studies should investigate deeper into specific abnormal features to increase understanding of their association with adverse outcome.

Although our assessment scheme has shown great potential for predicting 2-year neurodevelopmental outcome, there was no significant indicator between the EEG results and a particular Bayley III domain subscale. Furthermore, although objective, the assessment scheme is still dependent on interpretation by specially trained EEG reviewers. Future studies could make the assessment scheme more accessible for non-experts, or explore an automated system similar to those available for the term EEG (374, 471-473). Alternatively, exploring other physiological signals, such as HR and SpO2 in chapter 3, along with serial EEG monitoring in the NICU may provide further improvements for the prediction of outcome. Monitoring at all time-points for all infants was not always possible, with missing data especially evident at 12 hours, 72 hours and 35 weeks, which may have had a negative impact on the analysis. Late EEG application after 12 hours was mainly due to late consenting, or stability of the infant, while early removal of the EEG was mainly due to the clinician or parents' request. Additionally, the EEG-35 recording was occasionally missed due to early discharge or due to a transfer to another hospital. The Bayley III scores allowed for standardised assessment of all surviving infants but there are some well documented limitations of this test (343, 474). We used a cut-off score of 85 to ensure that infants with borderline abnormal results were identified and entered into a longer term 5 year follow up programme. It is also possible the Bayley III assessment at 2 years of age might have underestimated possible developmental issues (474) and that later testing at school age may reveal cognitive problems.

A large amount of EEG data was collected during the lengthy NICU stay of very preterm infants. Experienced neonatal electroencephalographers reviewed the recordings anonymously, blinded to clinical information and were not involved in the clinical care of the baby. We analysed the multichannel EEG rather than the aEEG because of the more detailed temporal and spatial information available (392). The aEEG is very limited for the accurate assessment of brain function in preterm infants because of the inability to identify specific waveforms, lack of spatial information and because of heavy filtering of the most prominent preterm EEG activity (475). In addition, in chapter 5, the assessment scheme was tested for inter-rater reliability between two specialists in the field of neonatal EEG. It demonstrated its usefulness in a clinical environment, therefore it might have the potential to be used in other centres. The effect of medication was considered during interpretation of the EEG, as drugs such as AEDs and morphine can supress the EEG and increase discontinuity. It was also essential to integrate EEG data with clinical information to aid investigation and prediction of adverse neurodevelopmental outcome in a clinical setting (476). This led to some uncertainty as it is difficult to prove how much of the changes are due to medication or due to an acute injury. We sought to minimise the influence of medication on EEG grading by annotating the timing of drug administration and considering the half-life of the drug. Although the core of the study concentrated on EEG monitoring, it was also essential to integrate these findings with the infants' clinical course. As preterm infants remain in the NICU for a prolonged period, they may develop complications, such as infections (476), and it is important to integrate the EEG data with the clinical data to aid the investigation and prediction of adverse neurodevelopmental outcome.

In conclusion, we report that multichannel EEG in preterm infants can be a very useful tool for predicting neurodevelopmental outcome at 2-years of age. The 35-week EEG proved to be the most accurate. A normal EEG at 32-weeks and 35-weeks were excellent indicators of normal neurodevelopmental outcome at 2-years, while an abnormal EEG highlights the potential for an abnormal outcome. Early EEG monitoring in the first 72 hours can also provide a useful baseline for early brain development. Comparing EEG and CRUS results showed that an EEG during any of the three postnatal periods was more predictive of outcome than CRUS. EEG should be considered as a useful adjunct to provide an indication of early brain development or to identify infants in need of further investigations such as MRI, ahead of discharge from the NICU.

Chapter 8. Discussion

Preterm infants are at high risk of complications such as brain injury and future adverse development. The clinical management of these infants in the NICU is challenging. It is therefore important to improve the quality of care to minimise this risk of complications. Improvements such as early prediction of outcome could potentially provide essential clinical information for early intervention, clinical management, and implementation of long-term support, where necessary. Consequently, the focus of my PhD was to study and assess the brain activity of preterm infants using continuous, multichannel EEG. To achieve this, I collected a cohort of EEG recordings from preterm infants during the early postnatal hours and days after birth.

8.1. Summary of main findings

The main finding from this thesis that make a significant contribution to current literature, is that EEG has the potential to accurately predict neurodevelopment at 2 years of age. Our findings show that by using a newly developed EEG assessment scheme of serial recordings or by using early multimodal physiological monitoring, we can predict poor neurodevelopmental outcome with high accuracy. Another significant finding from this thesis is that seizure frequency in preterm infants using continuous, multichannel EEG is substantially lower than results from previously reported aEEG studies. An additional finding from the thesis is the genetic influence on EEG concordance of MCDA twins in the early preterm period. The significance of these findings demonstrates the utility of EEG recordings in preterm infants.

In result **chapter 3**, multimodal simultaneous, physiological signals (EEG, SpO_2 and HR) were collected from the first days after birth. EEG grades were grouped into two categories: 1 = normal or mildly abnormal and 2= moderately or severe abnormal, while quantitative features of the SpO_2 (mean) and HR (skew) were also included in the model. Forty-three infants were classified as either at high or low risk of later morbidity based on their clinical course score and Bayley Scales of Infant Development-III was used to determine neurodevelopmental status at 2 years of age. Twenty-seven had normal outcomes, while 16 infants had poor outcomes or died. Results showed that quantitative analysis of physiological signals, combined with GA and graded EEG, has the potential for predicting mortality or delayed neurodevelopment at 2 years of age. Further studies are necessary to demonstrate the added benefit of the multimodal approach over the EEG alone however, as the improvement in performance failed to reach statistical significance.

A key role of the multichannel EEG is seizure detection and this remains the gold standard approach in neonates. Seizure frequency in the first few days of life is unclear in preterm infants. Therefore, we recorded continuous, long-duration video-EEG monitoring within the first three days after birth in a population of infants < 32 weeks GA **(chapter 4)**. In total, 120 infants and 6,932 hours of EEG were visually analysed, with only 6 infants (5%) developing a combined 307 electrographic seizures. This incidence rate is lower than what aEEG studies are currently reporting, suggesting that reviewing raw multichannel EEG is important for accuracy and that aEEG may be unreliable for seizure detection in preterm infants.

In addition to seizure detection, the EEG is also the gold standard for assessing background brain activity. Previous literature has suggested that analysis of background EEG patterns of preterm infants could predict neurodevelopmental outcome. However, there is no standardised method to evaluate and grade EEG in preterm infants. Therefore, the aim of **chapter 5** was to develop and assess the efficacy of an objective, age-specific EEG assessment scheme to evaluate and grade normal and abnormal EEG features in preterm infants. This scheme consists of four EEG categories; namely, temporal organisation, normal waves, abnormal waves and abnormal features, while the scheme also divides into six different groups of PMA, ranging from 23 – 36 weeks. Experienced reviewers who were not involved in the initial development of the scheme, graded EEGs to test the scheme and good agreement was obtained in all patients and EEG feature categories. This scheme was used in the next two chapters.

This assessment scheme assessed EEG similarities between preterm twins and age matched singletons in the first 72 hours of age, 32 weeks PCA and at 35 weeks PCA **(chapter 6)**.

Furthermore, EEG similarities were analysed between MCDA and DCDA twins. In addition to visual analysis, quantitative EEG analysis was undertaken where intra-class correlations were generated to estimate within twin similarities and compare similarities within MCDA and DCDA twins. Quantitative results showed strong correlations for twins and no correlations for singletons. Further investigation showed stronger correlations for MCDA twins in comparison to DCDA twins. Visual analysis was not as effective as the quantitative analysis, suggesting that EEG correlations are very subtle and can only be seen using detailed mathematical analysis. The main finding was that correlations were evident across all time-points in MCDA twins, supporting current knowledge relating genetic influences of the developing brain to the maturing patterns of the EEG.

Maturation of the serial EEG has previously been studied, however, we report the performance of our proposed assessment scheme for predicting adverse outcome from using three different EEG time-points **(chapter 7)**. Multichannel EEG recordings were undertaken in the first 72 hours after birth, at 32 weeks and 35 weeks PCA, while neurodevelopmental assessment was undertaken at 2 years of age. This neurodevelopmental assessment was obtained in 57 infants, 40 of which had normal outcome, the remaining 17 were diagnosed with abnormal outcome. Normal or mildly abnormal EEG recordings at approximately 35 weeks GA led to normal outcome in every case, while no infant with abnormal outcome at two years showed a normal EEG recording at this 35-week time-point. Maturational changes were also identified over the course of the serial recordings, with good outcome identified in every case where the EEG improved from the first to the last recording, in addition to the one case of EEG deteriorated showing a poor neurodevelopmental outcome. This suggests that not only is the EEG predictive, and that the use of EEG could be clinically useful before discharge, but also that using this specific assessment scheme is an efficient way to perform this prediction.

8.2. Significance of findings and contribution to literature

Various results were gathered during this study and numerous findings can contribute significantly to the current literature. In this thesis, I have reported the first study to analyse EEG in combination with GA and multimodal physiological signals, recorded within the first

72 hours after birth, to predict death or neurodevelopmental delay at 2 years of age. This highlights the potential value of multimodal monitoring and its possible role in predicting outcome during the early transitional period. Useful information collected from the model could potentially assist neonatologists in the NICU and guide early treatment strategies. Previous studies from Medlock *et al.(351),* Broitman *et al.(353)* and Tyson *et al.(354)* reported that multivariate models of early clinical information performed well in the prediction of outcome, however no model considered physiological measurements. Another study by Saria *et al.* showed that a model of early physiological quantitative measurements, including HR, RR, and SpO2, could predict short-term outcome with a high level of accuracy (355). Although physiological measurements were incorporated, brain activity was not considered. This study is the first to examine physiological quantitative measurements, including brain activity, in preterm infants, during the first days of life, to predict 2-year neurodevelopmental outcome. Although the clinical course score sensitivity was higher than the model, the specificity was lower. This is understandable as the clinical course scores were collected at discharge, following diagnosis of any major complications, while the model only had early physiological information from the first day following birth. The fact that the model had a larger AUC and higher specificity compared to information from discharge, is extremely encouraging that early information can aid clinical management. This also highlights the importance of clinical course considerations in the investigations of preterm infants. Due to the number of potential complications, it is important to look at whether adverse events occurred which may have impacted short-term development, and ultimately neurodevelopmental outcome.

To my knowledge, the preterm seizure study is the first to use continuous, long duration, video-EEG monitoring to qualitatively and quantitatively describe electrographic seizures in a large cohort of preterm infants <32 weeks during the early postnatal period. This study certainly contributes to the understanding of electroclinical and electrographic seizures in preterm infants. Previous aEEG studies have been reported during the early postnatal period, however seizure frequency ranged from 22- 48% (314, 315, 318). This is in contrast to the few EEG studies that performed short duration recordings, or were limited to infants with risk factors for seizures only (307, 324, 325, 384). These studies range from 0.9 – 8.7% in seizure frequency, thus highlighting the inconsistencies between aEEG and EEG studies when examining seizures in preterm infants. As the first study to report a large cohort of infants using continuous, long duration, video-EEG monitoring, it is possible to state that only 5% of our population had seizures. This is a much lower frequency compared to results from using aEEG studies, highlighting that misinterpretation is possible during aEEG recordings. Ultimately, preterm infants might be misdiagnosed and treated unnecessarily. Phenobarbitone and other AED therapy have a neurotoxic effect on the brain, therefore, multichannel EEG monitoring is essential to reliably diagnose and treat seizures as soon as possible, to restore baseline electrical patterns and to avoid treating infants unnecessarily. The difficulties in maintaining and interpreting multichannel EEG unfortunately continues to be challenging for NICU staff, therefore improved training and support is necessary. Early, multichannel, video-EEG should be considered in preterm infants when seizures are a concern, as it provides detailed information about the preterm brain and helps identify seizures especially when aEEGs are inconclusive. The results suggest that preterm infants of lower gestation, with low Apgar scores, higher CRIB II scores, and evidence of brain injury on CRUS, are the infants at higher risk of seizures. In turn, the use of multichannel EEG in preterm infants in the NICU setting may help prevent unnecessary AED treatment and consequently preventing potential neurotoxic effects.

The first tailored, age-specific preterm EEG assessment scheme is developed in this thesis, which specifically evaluates EEG activity from preterm infants at different PMA. Previous studies developed a glossary (175) and a standard computer-based system for EEG assessment and reporting (403). However, to best of my knowledge, this is the first scheme that defines normal and abnormal features in order to correctly grade the preterm EEG. This has also identified clearer boundaries between normal and abnormal features, useful in a clinical setting, although there is still room for improvement. This scheme can be used as a bench mark for future studies to test and validate with a larger sample size across multiple research centres. This is therefore a significant study, presenting a scheme that standardises the approach for preterm EEG grading, an area that has previously shown uncertainties and I believe that this approach is a simpler and clearer way for EEG readers to evaluate complex preterm EEG recordings.

The preterm twin EEG study adds to the scientific literature in a number of ways. This is the first study to investigate within-twin pair and between-twin group differences in a cohort of preterm twins < 32 weeks GA, at three different time-points within the postnatal period. There are no studies that attempt to describe EEGs in preterm twins, especially not as early as described here. The sample size is small, but this represents a significant effort in recruitment over a period of 13 months in a tertiary hospital. To collect synchronised EEGs from preterm twins is difficult in itself, however to only include infants without IUGR, grade 3 or 4 IVH, congenital anomaly, or who died during the NICU stay, highlights the difficulty to collect a larger sample size. A previous study (434) examined sleep EEGs from 60 healthy, near-term twins, however mean absolute difference was only analysed for spectral power, while this prospective study analyses multiple (22) features, including spectral power, of the EEG. Strong correlations between MCDA twins was found in many EEG features, especially with discontinuity and symmetry EEG features. Novel findings from this study demonstrate the genetic influence on the EEG even during this early stage in brain development.

EEG recordings from cohort 1 were used to develop the assessment scheme, while EEGs from cohort 2 were used in the final study, which assessed the performance of the newly developed scheme. The final study reports the first use of the scheme during serial multichannel EEG recordings in preterm infants for the prediction of neurodevelopmental outcome at 2 years of age. It has already been reported that EEG analysis has the potential to provide useful information for neurodevelopment outcome. Using the proposed assessment scheme supports these results and further identified that a pre-discharge recording at 35 weeks GA proved to be the most accurate period for prognosis. Previous studies have used serial EEG abnormality to predict outcome in very preterm infants, however this is the first study to record continuous multichannel recordings along with serial EEG recordings, using a tailored preterm EEG assessment scheme. A finding that contributes to the current literature was that 44% of the infants with an abnormal EEG-1 improved by the pre-discharge recording, and all of these infants had a good 2-year outcome. This pattern was not examined in chapter 3, therefore, further research is needed, however it does confirm that the EEG can improve following complications in the early postnatal period and lead to a positive outcome. This highlights that a pre-discharge recording is the best indicator while the earlier EEG would provide a baseline for early brain development which, of course, may be influenced by clinical events during the neonatal period in the NICU.

There were some common findings between chapter 3, chapter 7 and previous papers evaluating usefulness of preterm EEG and outcome. Both the multimodal analysis and the assessment scheme in chapter 7 showed higher AUCs than the clinical scores from the respective studies. This indicates that early EEG monitoring is more useful than a clinical score at discharge, by presenting useful information to clinicians during a vulnerable period, where monitoring progress and development is pivotal. Both chapters identified that dysmature and disorganised activity impacted normal outcome, similarly to Le Bihannic et al. (307), who also reported similar sensitivity and specificity of 83.3% and 88%, respectively. Differences were evident between chapters 3 and 7 however, with chapter 3 reporting higher specificity while chapter 7 reported higher sensitivity. This could be due to the different cohort of infants used, the fact that a different grading approach was applied, or that chapter 3 used one-hour epoch compared to two-hour epochs in chapter 7.

<u>Strengths of thesis</u>

An important element of the work within this thesis is the protocol and procedure for recording EEG data from vulnerable infants at such an early time-point in their lives and for a long period of time. In preparation of recruiting the second cohort, we developed a more practical method for EEG application to minimise the handling of preterm infants, while ensuring recording quality remained high. An efficient and effective method was developed, which consequently became our standard application procedure for all infants in need of EEG monitoring. Both cohorts were recruited from the Cork University Maternity Hospital, under specific enrolment criteria, following informed parental consent. Video-multichannel EEG was always recorded, which facilitated reviewing of suspicious EEG activity, thus enabling discrimination of genuine brain activity or artefact. Additionally, it was also important to distinguish clinical and electroclinical seizure characteristics. We incorporated the Bayley Scales of Infant Development III test at 2 years of age into two of our investigations. The assessments from the first cohort were undertaken by a specialist neonatal physiotherapist, while the second cohort was undertaken by the same specialist neonatal physiotherapist, a research psychologist and an occupational therapist. The results were anonymised (with a research code), stored on a secure database and were not accessed until the necessary analysis was needed, preventing bias during the EEG analysis.

To ensure an accurate frequency rate for seizures in chapter 4, all EEG recordings from 120 infants from both cohorts were visually analysed from the start of monitoring to the end of monitoring. In total, 6,932 hours of recording was examined, which is in stark contrast to current literature, as all preterm multichannel EEG studies only recorded for a small period of time. To our current knowledge, we have reported the largest combined duration of preterm EEG for the analysis of seizures in preterm infants. The main finding that only 5% of infants had seizures is an important result. This identifies a possible problem with the current application of aEEG in preterm infants. Recommendations have been reported in this study which hopefully will influence clinicians world-wide and hopefully influence more support and training for neonatal staff, as currently it would be very difficult for most neonatal units to implement multichannel, long term EEG monitoring on a day to day basis. It is a testament to the study that an editorial report has been published from Stanford University (477), highlighting the importance of the findings from this study while reiterating the importance of an appropriate approach to EEG monitoring in preterm infants.

In addition to seizure identification, using a standardised and tested assessment scheme is also important to identify normality of the background activity of preterm infants. A developed assessment scheme was used for the investigation in chapter 6, in addition to chapter 7. This scheme was established following an extensive literature review of normative physiological and pathological features in order to objectively evaluate the EEG according to the specific GA. This scheme showed predictive ability with statistically significant results for adverse outcome at 2 years, which could prove to be beneficial in the clinical setting. This will not only assist clinical management of the infant, it will also help clinicians answer questions from parents, provide them with updates and offer information to prepare them for future scenarios.

This scheme was also used when analysing synchronous EEG in preterm twins. A study analysing the EEG within preterm twin pairs, during synchronous EEG recordings is novel and has never been attempted before. EEG studies of children and full-term twins have been studied, but none of which included preterm infants at such an early time-period. In addition to this, not only was the EEG visually analysed, we also used quantitative analysis of numerous EEG features to investigate the similarities within the twins. Identifying that mathematical analysis was successful in showing EEG concordance in MCDA twins is a significant finding, as quantitative analysis could have been easily overlooked as visual EEG analysis is more accessible and is a more standardised approach of analysing EEG recordings.

Limitations of thesis

The primary limitation of this thesis is the small sample size. Data was collected over two recruitment periods, however due to the different inclusion criteria for each chapter, it was only in chapter 4 that both cohorts could be combined and work with a larger sample size. Due to specific guidelines of inclusion criteria for each study, several EEG recordings were excluded due to small recording durations, late EEG application, missing serial EEG recordings, missing physiological data or missing follow-up neurodevelopmental outcome data. Additionally, in chapter 6, a small sample size of preterm twins was collected, although this was the first known investigation for the EEG activity of preterm twins. The focus in both recruitment periods was to apply the EEG as soon as possible after birth, however the clinical aim was to stabilise the infant, therefore early application was not always appropriate. Other reasons which affected EEG application was lack of EEG machines, lack of EEG staff (especially during recruitment of the first cohort) or declining of consent. The main reason behind declining consent was to minimise handling of infants.

A consequence of small numbers is that it limits multivariate analysis (as in chapter 3 and 7), as the number of explanatory variables were limited to avoid over fitting in the models. With larger sample sizes other clinical factors such as respiration, blood pressure, initial pH, lactate, and Apgar, could be explored as confounding variables. However, although sample size is limited, it has allowed novel work such as a multivariate model for outcome prediction, investigation for EEG activity in preterm twins and investigation into a new preterm EEG assessment scheme to be undertaken.

Another noticeable limitation is the potentially subjective nature of EEG interpretation, even when utilising an assessment scheme. The development of an automated system would improve upon this limitation for future studies. An automated grading system could be developed for preterm EEG similar to available systems for hypoxic-ischaemic encephalopathy in the EEG of term infants (374). Although early EEG provides an insight into the current condition of the infant during the early transitional period, the infant could face later challenges and complications that could impact their long-term outcome. Serial EEGs and physiological measurements over the infant's stay in the NICU could add additional predictive information and provide early indications at the beginning of critical care in the NICU (307).

A general limitation is that only short EEG epochs of 1 hour (in chapter 3) or 2 hours (in chapter 5, 6 and 7) were used in the EEG analysis. This was enough to discover the temporal organisation, such as state change, and it also gave a large enough period to assess normal and abnormal activity; however, there were periods of the EEG that were not analysed. The methodology chosen was due to time constraints of analysis. This is a common limitation in long term EEG studies, but necessary to ensure accurate EEG interpretation. The use of trends, such as aEEG and compressed spectral array or seizure detection algorithms, can assist and possibly speed-up analysis; visual analysis of every epoch, however, remains the most consistent approach to analyse EEG. This approach was undertaken in the seizure incidence study where every epoch of 6,932 hours of EEG recordings were reviewed for seizures. This extremely time-consuming process was important to ensure accurate seizure identification.

A limitation to chapter 7 was that physiological signals from chapter 3 (HR and SpO_2) were not included in the prediction of neurodevelopmental outcome. These data were collected when available, however this was often not retrievable as certain Philips IntelliVue MP70 monitors were not configured to simultaneously integrate the data to the EEG machines. This issue was highlighted on weekends when moving monitors from one cot-side to another was not possible.

Findings from this thesis should provide a degree of reassurance for clinicians. A thorough, tested assessment scheme has been proposed, that is easy to use. This provides encouragement for clinicians, that useful information can be gathered from EEG at an early stage, if needed. Although the debate will continue, the significant finding of lower seizure frequency in preterm infants should be kept in mind, as ultimately unnecessary treatment may cause more damage.

8.3. Future recommendations

The validation of the preterm EEG assessment scheme and results presented in all chapters are key advancements for the future of preterm EEG. The assessment scheme has proven effective for predicting outcome in our second cohort in chapter 7, however it should be validated with multi-centre data by researchers and clinicians who were not involved in its development. Furthermore, exploring physiological signals, such as HR and SpO2, along with serial EEG monitoring in the NICU and for further improvements of the EEG assessment scheme, might prove to be useful. This would ensure that our findings truly reflect its accuracy, increasing its potential in the clinical setting. In terms of further modifications to the scheme, maybe an option to mark features as 'inconclusive' would be beneficial. In one situation during testing, the experts had to score STOPS/Occipital sawtooth in an extremely abnormal EEG recording. It was very difficult to identify the presence of such a subtle feature in such an abnormal EEG recording. Instead of being doubtful and obliged to answer present or absent, an option to express uncertainty would be useful and may provide a more accurate score. An automated grading system similar to the available system for hypoxic-ischaemic encephalopathy in the EEG of term infants would be beneficial (471). This would enable continuous long-term monitoring to aid the clinician's judgement of the infant's clinical condition and possible prognosis. Although the development and validation of an algorithm is difficult and time consuming, it is not necessarily out of the question that adaptations to current algorithms may be possible to avoid a significant delay in development.

Furthermore, developing a preterm seizure algorithm could also be achieved to remove the dependence of the reviewer's possible subjective interpretation. Preterm seizures are different and harder to detect compared to full term seizures, especially when using aEEG, therefore a specific seizure detection algorithm for preterm infants would be advantageous. The results from chapter 4 are not definitive, therefore a multi-centre study with multiple EEG examiners with an even larger cohort of infants would be the next step in future research before an algorithm could be devised. In addition, for future studies, I would recommend the involvement of two examiners; an experienced EEG user designated to identify seizures via multichannel EEG and a designated, experienced aEEG user to identify seizures via the aEEG. Interrater agreement could be calculated, pinpointing disagreements between the users, possibly highlighting consistent, repeating misinterpretations.

A similar approach would be useful for the understanding of EEG in preterm twins. A better understanding would enhance monitoring of these infants in the NICU. If we can confirm that EEGs in MCDA twins are similar, it might enhance the ability to understand and diagnose individuals based on the brain activity of their twin. A larger sample size is needed and a multicentre study again would be extremely advantageous in future research, due to the difficulty of gathering the data from one site alone. The same quantitative analysis approach can be applied to all sites to ensure consistent, objective results. Another possible modification to future work approach would be to consider sleep staging. Although time-locked and synchronous EEGs were recorded within the twin pairs, a limitation of this is that this does not necessarily mean that both infants are in the same time-locked sleep state. Taking this into consideration, future studies should consider time-locked, synchronous, long term monitoring at all three time-points, as per Chapter 7. Instead of analysing particular, fixed epochs (such as 12, 24, 48, 72 hours), it might be helpful to visually review the time-locked EEGs and identify periods where the same sleep stage is witnessed in both infants, and analyse these periods. The limitation of this, however, would be the inconsistent post-natal age between the twin pairs.

This thesis has taken an important step forward in the advancement of preterm EEG monitoring. Valuable results and conclusions have been published and the next steps are to validate the work further for future clinical use.

Personal reflection following the thesis

The development of this thesis to its ultimate conclusion has been a constant source of revelation in so many ways, both in highlighting the existence of often unappreciated values of human decency, but also in opening my eyes to a whole range of issues previously unconsidered. Unforeseen challenges have arisen and have had to be confronted, a process which has provided an invaluable life experience and which will hopefully stand me in good stead in the years that lie ahead.

Working in the intense atmosphere of the NICU has only served to underline for me the extraordinary professionalism and dedication of the clinicians and nursing staff there, and reinforced the utmost respect I have for their calling and endeavours. Throughout the entire journey of this PhD, the CUMH staff members have been a source of constant support. Integral to the nature of my work was the essential need to monitor and rigorously observe the development of all the infants throughout their entire stay in the unit, and I am indebted to the conscientiousness of staff members regularly providing updates of news or any changes in the wellbeing of those infants. Such cooperation demonstrates the strong multidisciplinary teamwork which is such a powerful hallmark of our research centre. Furthermore, the fact of having been granted the opportunity to be so heavily involved in the clinical care of these preterm infants, and also in research that may hopefully lead to a more enhanced future for the prematurely born, has been a very humbling and fulfilling experience.

Fundamental to a study such as this is the nature of human interaction. As great a debt of gratitude that is owing to the professionals of CUMH, nothing could have been achieved without the cooperation of so many parents, themselves often in a state of anxiety and distress. Initial meetings were always at a time when the parents themselves were under considerable stress following the unexpected delivery of their vulnerable and premature baby. At such a time of diverse emotions, any approach to elicit their participation in the study had to be handled with compassion and extreme sensitivity. The fact that so many naturally apprehensive parents were so generous and accommodating in the interests of research is something that will forever remain with me.

On a personal level, the production of this thesis has generated both demanding tasks and unforeseen issues which have had to be overcome, but these in turn have equally served to promote skills which will undoubtedly prove of immense benefit to me in the future. Opportunities to engage with peers at regular departmental meetings and to meet with contemporaries and speak at international conferences have increased my self-confidence and enhanced my presentational expertise. Research skills have been advanced and honed, as hypotheses have had to be developed in the face of new ideas, and revision of current and relevant literature has had to be understood. The acquisition of a wide range of invaluable experiences will unquestionably serve me well in years to come.

Above all, though, the overriding emotion that will certainly remain with me, is how privileged I feel to have been granted the opportunity to be involved in the clinical care of these preterm infants, and in future research that may prove advantageous to other premature infants in future years.

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Appendices

APPENDIX A – PIEeg Ethical Approval

COISTE EITICE UM THAIGHDE CLINICIÚIL
Clinical Research Ethics Committee

UCC

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Coláiste na hOllscoile Corcaigh, Éire
University College Cork, Ireland

Lancaster Hall,
6 Little Hanover Street,
Cork,
Ireland.

Our ref: ECM 4 (m) 12/03/13

20th February 2013

Professor Geraldine Boylan
Professor of Neonatal Physiology
Department of Paediatrics & Child Health
Neonatal Brain Research Group
Room E25
5th Floor
Cork University Maternity Hospital
Wilton
Cork

Re: The role of EEG for the clinical management of preterm infants and the prediction of long term neurodevelopmental outcomes. EEG in Preterms – PIEeg.

Dear Professor Boylan

Expedited approval is granted to carry out the above study in:

- Cork University Maternity Hospital.

The following documents have been approved:

- Signed Application Form
- Study Protocol Version 1.0
- Informed Consent Form Version 1.0 dated 18th February 2013
- Parent Information Leaflet Version 1.0 dated 18th February 2013
- Cv for Chief Investigator.

We note that the co-investigators involved in this study will be:

- Professor Geraldine Boylan, Mr Rhodri Lloyd, Dr Peter Filan and Professor Tony Ryan.

Yours sincerely

Dr Michael Hyland
Chairman
Clinical Research Ethics Committee
of the Cork Teaching Hospitals

The Clinical Research Ethics Committee of the Cork Teaching Hospitals, UCC, is a recognised Ethics Committee under Regulation 7 of the European Communities (Clinical Trials on Medicinal Products for Human Use) Regulations 2004, and is authorised by the Department of Health and Children to carry out the ethical review of clinical trials of investigational medicinal products. The Committee is fully compliant with the Regulations as they relate to Ethics Committees and the conditions and principles of Good Clinical Practice.

APPENDIX B – PIEeg Protocol

<u>The role of EEG for the clinical management of preterm infants and the prediction of long term neurodevelopmental outcomes (short title: EEG in Preterms - PIEeg)</u>

Rhodri Lloyd

Chief Investigator:- Prof Geraldine Boylan

Co-Investigator:- Dr Peter Filan

Protocol Version 1.0

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Introduction

Electroencephalography (EEG) is the gold standard for monitoring electrical brain activity. The role of EEG, in the neonatal intensive care setting, is well established in the care of term newborn infants with neonatal encephalopathy. EEG has proven central to the assessment and evolution of the degree of encephalopathy, the management of neonatal seizures and has proven a reliable prognostic tool in relation to future developmental outcomes (Murray et al 2009).

Preterm birth (birth gestation < 37 weeks) is a growing healthcare, societal and economic issue internationally. The incidence varies from 5% to up to 13% in some countries e.g the United States. Preterm birth is associated with a risk of long term neurodisability which is inversely related to gestation and birth weight (Moore et al 2012). There is growing knowledge of infants EEG, but there is limited experience of its clinical role and whether it has a role in neurodevelopment prognosis. EEG or amplitude integrated EEG (aEEG) should now be regarded as a standard of care when caring for encephalopathic term infants (Boylan et al 2010). The majority of the literature concentrates on the amplitude-intergrated EEG (aEEG) and very little looks at continuous EEG monitoring. The EEG of a normal infant born at a GA of < 28 weeks is known to be discontinuous, comprising of periods of bursting activity alternating with interburst intervals (Selton et al 2002). These interburst intervals appear at much lower amplitudes to the active burst and can last for up to 45 seconds/1 minute. The bursts themselves can be very brief, lasting only 1 second, but can also last as long as 3 minutes. These values are very subjective with different literature suggesting a different range in normal values.

Seizures in these infants are thought to have an association with cerebral pathology and occur most commonly in the first 72 hours of life. It has been suggested that the occurrence of seizures are associated with many increased risks of neurodisability and mortality (Pisani et al 2012).

Clinical recognition of neonatal seizures is difficult; there are nonspecific involuntary movements which are over-diagnosed, whilst there are true subtle seizure manifestations which often go unnoticed. The use of continuous video-EEG monitoring in the Neonatal

Intensive Care Unit (NICU) can accurately identify the presence of ongoing subtle clinical and subclinical seizure activity, which health professionals often miss in the busy NICU environment.

There is knowledge on the maturity of EEG characteristics in full term infants, however this is not the case in the preterm infants. The knowledge of how the EEG evolves during the post natal period is very limited. The electrophysiological brain activity could mature differently in different individuals and could
depend on the gestational age of the infant, therefore individual EEG traces could evolve completely differently depending on the birth age. A study by Wikstrom et al 2012 showed that the evolution of the EEG in the first 24 hours can be of use, as low voltage activity or burst suppression can be predictive of the high possibility of a poor developmental outcome. It is clear from this study that early electrophysiological recordings can be a useful tool in predicting the outcome in preterm infants.

Aim

This study aims to establish the most useful EEG characteristics in the very preterm infant (<32 weeks gestational age (GA)) that determine long term outcome at 2 years of age. We will investigate the potential role of EEG in clinical management and describe the incidence of electrical seizures in these young infants. Characteristics of electrographic seizures and seizure burden in preterm infants is not well established, therefore the findings could correlate with a two year neurodevelopmental outcome. Continuous EEG monitoring is the most valuable tool for the detection of brain activity in infants, including seizure capture and the evolution of activity therefore by looking at an evolving trace, we can try and analyse whether certain characteristics are more predictive of a poor prognosis.

Eligibility Criteria:-

Eligible Patients:- Preterm infants less than 32weeks GA will be recruited from the Neonatal Intensive Care Unit (NICU) at Cork University Maternity Hospital (CUMH) over a 2 year period. Written informed antenatal or postnatal consent will be obtained from the parents of each infant studied.

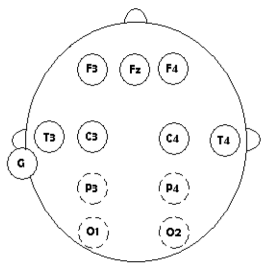
Inclusion Criteria:- The inclusion criteria will involve infants below 32 weeks GA with no known congenital anomalies.

Exclusion Criteria:- The exclusion criteria will involve infants below 32 weeks GA with known congenital anomalies that are likely to affect future long term development.

Method

Continuous, multi-channel, non-invasive EEG monitoring will be commenced as soon as possible after birth once consent has been obtained using NicoletOne EEG machine. The EEG machine also provides two a-EEG channels from the electrodes placed. The international 10-20 measuring system, modified for neonates, will be followed for application of 10 EEG surface electrodes. Ideally 10 electrodes will be placed - F3, F4, C3, C4, T3, T4, O1, O2, Ground (left mastoid preferably,) and Reference (Fz). However, this may sometimes be difficult to achieve particularly if the infant is not handling well or access to the scalp is restricted. In this case the O1 and O2 electrodes may be positioned at areas P3 and P4. Subsequently, the minimum electrodes placed will be F3, F4, C3, C4, P3, P4, Ground and Reference (Fz) (Fig 1). ECG and respiration monitoring are also included in a routine EEG investigation where ECG leads are placed across both shoulders and a respiratory monitor is placed near the chest. A 2 year neurodevelopmental follow up will be performed using the Bailey III neurodevelopmental assessment method.

Figure 1 Showing placement of scalp EEG electrodes



Due to the fact that preterm infants are fragile and need to be at a controlled temperature, the application of the EEG electrodes must be completed efficiently and quickly in approximately 10 minutes. The initial EEG recording will last for approximately 72 hours, while the repeat EEG will last between 4 and 6 hours. If the attending clinician requests an additional EEG due to an acute illness, the EEG will be recorded for approximately 24 hours. A Clinical Physiologist and Neurophysiologist will interpret the EEG tracings.

Step by step process of recruiting data

- Approach the parents and ask them to read the Information Leaflet.
- Present the parents with the Informed Consent Form
- Obtain written informed consent from both parents or guardian. This may be obtained either antenatally or postnatally
- Observational, non-invasive, multichannel EEG monitoring will commence, as soon as possible after birth and will continue for up to 72 hours.
- All clinical and demographic details will be recorded and stored securely in an encrypted data server.
- Cranial Ultrasound imaging data will also be collated and stored if performed.
- A repeat EEG will be performed at 32 weeks GA, and will be recorded for between 4 and 6 hours.
- Another EEG will be performed pre-discharge or at 36 weeks GA, and will be recorded for between 4 and 6 hours.
- An extra 24 hour EEG will be performed, when requested by the attending clinician. This could be requested at any point during the infants stay in the unit. Circumstances which could lead to this request involve the following acute illnesses:-
    - Recognition of Intraventricular Haemorrhage
    - Sepsis
    - Pulmonary Haemorrhage
    - Necrotizing Enterocolitis
    - The need for reintubation
- All infants born less than 32 weeks GA routinely have a Bayley neurodevelopmental assessment at full term corrected age in CUMH.
- The results of the neurodevelopmental assessments will be collated for each baby recruited and correlated with early EEG findings, including seizure severity.

Sample Size

This is an observational study rather than an interventional study, therefore the sample size is based on expected infants. An annual report in 2011 showed that 83 infants less than 32 weeks of gestation were admitted to the Neonatal Intensive Care Unit, whilst there were 103 admissions in 2010.

The consent rate of local studies estimates approximately an 80% success rate therefore, a prospective cohort study of 80 - 120 preterm newborn infants born <32 weeks of gestation will be obtained over a 2 year period.

Data Storage

The study protocol will be reviewed and approved by the Cork Research Ethics Committee (CREC). All study personnel will implement the clinical investigation with full respect and compliance of the legal and ethical European / institutional requirements and codes of practices. All data will be saved and the patient's details will be kept anonymous within the department. The UCC server and an encrypted drive will be used to store the results and data through a secure computer.

Conclusion

This research project will strive to establish the most useful EEG characteristics in the very preterm infant (<32 weeks GA) that may accurately predict long term outcome at 2 years. We also aim to establish the impact of early postnatal seizures in very preterm neonates by correlating with neurodevelopmental outcome at 2 years. This project will investigate the possible role of EEG in clinical management of preterm infants and their long term prognosis.

Co-Investigators

Prof Geraldine Boylan, Professor of Neonatal Physiology, Department of Paediatrics' and Child Health

Mr Rhodri Lloyd, (Clinical Physiologist (Neurophysiology)), PIEeg Project Researcher/PhD student, Department of Paediatrics' and Child Health

Dr Peter Filan Prof Tony Ryan Prof Eugene Dempsey Dr Brendan Murphy Dr Liam O'Connell	Consultant Neonatologists Department of Neonatology, Cork University Maternity Hospital.

Dr Niamh Lagan, Specialist Registrar in Paediatrics, Department of Neonatology, Cork University Maternity Hospital

Dr Darragh Finn, Specialist Registrar in Paediatrics, Department of Neonatology, Cork University Maternity Hospital

Mr Robert Goulding, (Specialist Clinical Physiologist (Neurophysiology)), NEOPRISM Project Researcher/PhD student, Department of Paediatrics' and Child Health

Dr Liudmila Kharoshankaya, Clinical Research Fellow, Department of Paediatrics' and Child Health

Dr Caroline Ahearne, Senior House Officer/Clinical Fellow, Department of Paediatrics and Child Health, Cork University Maternity Hospital

Potential Benefits and Risks

The study will collect valuable information that will help improve future treatment for premature infants. The preterm EEG is not understood well enough, therefore collecting information will benefit management of premature infants in the future. One of the main objectives of this research is to try and understand the preterm EEG better.

There are no side effects or risks from participating in this research study.

Reference:

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Wikstrom S, Pupp IH, Rosen I, Norman E, Fellman V, Ley D, Hellstrom-Westas L (2012) "Early single-channel aEEG/EEG predicts outcome in very preterm infants" Acta Pædiatrica, (7):719-26

APPENDIX C – PIEeg Parent Information Leaflet

<u>Parent Information Leaflet</u>

<u>The role of EEG for the clinical management of preterm infants and the prediction of long term neurodevelopmental outcomes (short title: EEG in Preterms - PIEeg)</u>

Your baby has been born before term or 37 weeks of pregnancy (premature) and has been admitted to the neonatal intensive care unit (NICU). You are being asked to take part in a research study that involves premature babies. We would like to discuss this study with you, so that you understand what it is about. If you decide to join the study you will be asked to sign an informed consent form, a copy of which you can keep in addition to this information leaflet.

A member of our research group will be available to answer any further questions you may have before you make a decision about taking part.

What is the study about?

Some babies are born early (prematurely) and may need to spend some time in the neonatal intensive care unit (NICU). During the time in the NICU a premature baby will grow and we are particularly interested in how the brain develops and how this relates to long term developmental outcome.

We use different tests to monitor brain development. One of the tests we use measures tiny electrical signals from the brain and is called an EEG (**e**lectro**e**ncephalo**g**raphy). EEG is very useful and routinely used to monitor full term babies in our unit. We need to establish if it is just as useful for premature babies.

This study aims to use EEG to monitor the electrical brain activity of premature babies. The EEG would be used for the first three days after your baby is born and repeated again approximately three times for short periods while your baby is in the NICU. We want to investigate if the EEG will help us understand how your baby's brain is behaving and developing. We also want to know if the EEG will help us to predict how well a premature baby will do later on in childhood.

The study will use other measures of the baby's health e.g. heart rate, breathing rate and blood oxygen levels. These are routinely recorded in all babies in the NICU. We plan to look at this information together with the EEG recordings.

What is an EEG?

An EEG is a specialised way of recording small electrical signals that are produced in the brain. These signals are measured using a series of small pads that are placed onto your baby's head. These pads do not give out any electrical activity or cause any pain to the baby - they only act as 'receivers' to pick up and display the signals on a screen beside the baby's cot. At the same time, your baby will have a video recording to monitor any movements, which can be very important when trying to understand the EEG. Once the EEG is complete the pads are removed.

What are your options?

Being part of the study is entirely voluntary. If you agree to take part we will ask you to sign an informed consent form, and you will be given a copy of this to keep. You are free to change your mind and withdraw your baby from the study at any time, without giving a reason. The care that you or your baby receive now or in the future will not be affected in any way by your decision whether or not to take part in this research study.

What will happen to my baby if I choose to allow my baby to take part in the study?

Your baby will have EEG monitoring for the first three days after birth. Your baby may also have EEG monitoring for short periods at three other times while still in the neonatal unit.

This EEG information will be collected in an anonymised database. Later on we will look at this information in greater detail and look for specific patterns or trends.
We will contact you at a later stage to ask if we can follow up your baby's development at two years of age.

Are there any possible disadvantages to taking part?

There are no disadvantages to taking part and it will not affect the care your baby receives.

What are the side effects of any treatment received when taking part?

There are no known side effects from participating in this research study.

What are the possible benefits of taking part?

We cannot say that taking part will be of any benefit to your baby but we hope the study will collect valuable information which will help improve future treatment for premature babies. We do not yet really understand what the preterm EEG is telling us and trying to better understand it is one of the main reasons we are performing this study.

What if there is a problem or I decide I don't want my baby to continue with the study?

If you have any concerns or you are not completely happy after giving consent then you can withdraw your baby from the research study at any time.

Will information about my baby be kept confidential?

Yes we will follow ethical and legal practice and all information about you and your baby will be anonymised and stored securely.

What if relevant new information becomes available?

You will be informed of any new information as it arises and if this changes your decision about taking part then you can withdraw your baby from the research study.

Will any genetic tests be performed?

No genetic tests will be performed.

Who is organising and funding the research?

The research team is led by Professor Geraldine Boylan (Director of Neonatal Brain Research Group, Chief Investigator) and includes Mr Rhodri Lloyd (Researcher), Dr Peter Filan (Consultant Neonatologist, Co-Investigator), and several other members from the Neonatal Brain Research Group, who you may meet during your time in the NICU.

This research study is funded by The Wellcome Trust which is a charity organisation. If you have further queries concerning your rights in connection with the research, you can contact the Clinical Research Ethics Committee of the Cork Teaching Hospitals, Lancaster Hall, 6 Little Hanover Street, Cork.

Who has reviewed this study?

The Clinical Research Ethics Committee of the Cork Teaching Hospitals has reviewed this study and they have approved the research protocol.

Expenses and payments

There will be no costs whatsoever to you and there will be no payment for taking part in this study. This study will be indemnified by the University College Cork research insurance policy.

Please complete the attached consent form ONLY if you are happy to participate in this research project. If you have any further questions please contact:

Rhodri Lloyd (021 420 5972, 086 450 6198)
Neonatal Brain Research Group,
Department of Paediatrics and Child Health,
University College Cork,
Cork University Maternity Hospital,
Wilton,
Cork

APPENDIX D – Informed Consent Form

NEONATAL BRAIN RESEARCH GROUP   π – EEG   Ospideal Máithreachais na h'Ollscoile Corcaigh / Cork University Maternity Hospital

PIEeg INFORMED CONSENT FORM

Title of Project: The role of EEG for the clinical management of preterm infants and the prediction of long term neurodevelopmental outcomes (short title: EEG in Preterms - PIEeg)
Name of Contact: Rhodri Lloyd (PIEeg Study Researcher)
Principal Investigator: Professor Geraldine Boylan **Co-Investigator:** Dr Peter Filan
Contact details: 021 420 5972 / 086 450 6198
Study Number: **Patient Identification Number for this trial:**

Please tick the box

1. I confirm that I have read and understand the Parent Information Leaflet and have been given a copy to keep. I have had time to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I agree that a recording of video-EEG can be performed on my baby. ☐
3. I understand that my taking part in the study is voluntary and I am free to withdraw my baby at any time without medical treatment being affected in any way. ☐
4. I agree that if I choose to withdraw my baby from the study I give the PIEeg research team permission to keep and use any data collected prior to this withdrawal. I understand that I can withdraw this permission at any time. ☐
5. I give permission for the study personnel to look at my own medical records and my baby's medical records for clinical and demographic details. I have been assured that information about me and my baby will be kept confidential and anonymised. ☐
6. I understand that ethical committees may look at my baby's or my own medical notes for audit purposes. ☐
7. I consent that anonymised data collected for this study may be used, now or in the future, for commercial or collaborative research to develop an automated system for monitoring brain health in newborn babies. ☐
8. I agree that video-EEG recordings, and physiological recordings obtained during this study, can be used by the Neonatal Brain Research Group and the Department of Paediatrics' and Child Health, University College Cork for training and research purposes. ☐
9. I agree to take part in a follow-up study at 24 months of age, that will assess my baby's development, and that I can be contacted to arrange this follow-up. ☐

Name of Parent/Guardian	Date	Signature
____________________	____________	____________________
Name of Parent/Guardian	Date	Signature
____________________	____________	____________________
Name of Researcher /Informant	Date	Signature
____________________	____________	____________________

18 FEB 2013 Version 1.0

APPENDIX E –

<u>Publications researching the prognostic values of aEEG/EEG in preterm infants.</u>

Author	*Year*	*Gestational Age*	*Number of infants*	*Aim*	*aEEG / EEG*	*Start of monitoring*	*Recording duration*	*Evaluation of development*	*Findings*
EEG									
Biagoni (192)	'00	27-34	40	Prognosis value of EEG abnormalities in PVL infants	EEG	Within 2-3 weeks or at term age, or both.	At least 40 minutes	Griffiths & Uzgiris-Hunt at 12 months	Dysmaturity more apparent with PVL and cognitive outcome. During early postnatal period – EEG is useful diagnostic tool for WMI in preterm infants.
Selton (409)	'00	26-28	17	EEG criteria of normality preterm infants at 26 – 28 CA	EEG	Most started within first 3 days.	At least 45 min or until two behavioural stages captured	Neuropsychomotor and sensorial evaluation at 2 years.	Normal EEG patterns in very preterm infants.

Maruyama (308)	'02	27 - 32	295 (46 with CP)	EEG to predict risk of Cerebral Palsy	EEG	First week	40-60 min	Psychomotor 18months	Grade of ASA on day 1 or 2 to predict outcome.
Okumura (280)	'02	<33	183	Existence of CSA to clarify their relation to neurodevelopmental outcome in preterm infants	EEG	Within 72 hours of birth	At least 40 minutes	Psychomotor development examined every 3 months after discharge until 18 months	Disorganised common to PVL and normal cognitive development. Dysmature EEG correlated to cognitive impairment.
Okumura (292)	'03	<32	31 PVL & 62 control	Clinical significance of abnormal sharp transients in PVL	EEG	Within 3 weeks of birth	At least 40 minutes during wakefulness and sleep	FU at 2 years to classify the infants into groups of different severity of diplegia.	Frontal & Occipital sharp waves may be useful for predicting PVL. OS associated with pathological findings/poor outcome. FS often closely related to deep white matter lesions.
Kato (217)	'04	<32	11	Investigate EEG aspects of Periventricular	EEG	Within a week of diagnosis	40min	FU not described (days -17 years of age)	ASA & CSA give valuable info for STO & LTO in preterms with PVHI

				haemorrhagic Infarction (IVH4)					
Pisani (478)	'07	Newborns	106 (51 preterm infants) with confirmed seizures	Compare neurodevelopmental consequences of recurrent seizures and status epilepticus	EEG	N/A	Monitoring of at least 60 minutes or until a complete cycle of states.	Neurologic follow up 44 weeks, 1 month after discharge, 3, 6, 9, 12, 18, 24 months.	Preterm infants with status epilepticus have high probability of future severe neurologic disability and postnatal epilepsy
Pisani (321)	'08	<36	835 PT births in this period. (51 with seizures)	Identify early predictors of poor neurodevelopmental outcome in preterms with seizures.	EEG	N/A	Monitoring of at least 60 minutes or a complete sleep cycle	Serial follow up to 30 months. Neuromotor, Amiel-Tison, Dubowitz exam & Griffiths mental developmental scale.	Preterm seizures associated to high rate of mortality & severe morbidity in survivors. Abnormal background EEG & Apgar 1 min predicts long term outcome.
Davis (326)	'10	<36 weeks	6499 included in the study. 414 had clinical seizures, 92 confirmed by EEG	Examine risk factors for seizures and determine the independent association with death/ neurodevelopmental impairment in preterms.	EEG	Discretion of clinician	N/A	Bayleys and Psychomotor development index – 18 to 22 months.	Infants with clinical seizures are at increased risk for adverse neurodevelopmental outcome, independent of multiple confounding factors.

West (305)	'11	<29	76	aEEG to predict outcome	2-channel EEG	EEG commenced within 48 hours of birth	Recorded for duration of 60 minutes.	18 months Bayley scales Assessment	EEG continuity a potential measure for identifying outcome.
Hayashi-Kurahashi (293)	'12	<34	333	Prognostic value of EEG in preterms	EEG	Within 72 hours of birth. The 3 serial EEGs; within 6 days, 7-19 days & 20-36 days.	40 minute recordings.	12-18 months Tsumori-Inage Infant Developmental Scale OR Kyoto Scale of Psychological Development.	Abnormalities in first month significantly predicts neurodevelopment at 12/18months. Mortality rate 6% in all live births & incidence of severe brain injury was 9% in survivors. PRS 2% of survivors & 20% of survivors with brain injury. Study showed that EEG assessment is better predictor than information from risk factors. A disorganised pattern without PRS is common and is an indicator of

									predicting adverse outcomes.
Le Bihannic (307)	‘12	<30	76 eligible – 61 included	Correlate EEG findings with the neurological/ neuropsychological outcomes.	EEG	First week of life. Repeats at 31/32weeks and 36 weeks.	Mean duration 60 min (min 45 minutes)	Clinical examination 4, 6, 9, 18 months, 2-4 years and 5-6 years.	Dysmature and disorganised EEG useful predictors of outcome.
Schumacher (310)	‘13	<31	41	Assess automated tABP for predicting outcome.	EEG	Within 12 hours of delivery	3 days	2 year Bayley scales Assessment & Peabody assessment	Low tABP potential to predict poor outcome in younger preterm infants.
Iyer (306)	‘15	22 - 28	43	Assessment of scale-free EEG bursts for predicting long-term outcome	2 channel EEG	At 12, 24, 48 and 72 hours	average duration of 115 min (range 70-120min)	2 year Bayley scales Assessment	EEG burst analysis, using the techniques of scale-free systems, shows potential for predicting long-term outcome in preterm infants
Perivier (309)	‘16	<32	1954 enrolled – 1744 follow-up – 1345 had at least one EEG	Assess EEG for predicting outcome using brain imaging and clinical assessment	EEG	First week	Minimum of 45 minutes	Brunet–Lézine Test and/or the Age and Stages Questionnaire at 2 years	Very abnormal EEGs with persistent or severe abnormalities predicted poorer developmental outcome. Severe abnormalities,

									included excessive discontinuity with maximal interburst interval duration above one and a half the maximal value for the corrected age, seizures, the absence of sleep cycles and positive rolandic sharp waves at more than1/min,
aEEG									
Hellstrom-Westas (276)	'01	<33	63	aEEG prediction of outcome in preterm infants with large IVH	aEEG	First week	At least 24 hours	Scheffzek classification, which categorises infants as neurodevelopmentally healthy or abnormal	In preterm infants with a haemorrhage, the number of bursts/hour in the aEEG trend during the first 48 hours is predictive of outcome

Wikstrom (303)	‘08	24 - 28	16	aEEG to predict outcome when affected by brain injury	aEEG	During the first 72 hours of life.	Median duration of 18.6 hours (3.0-55.3 h)	2 year Bayley scales Assessment	Early EEG depression associated with poor outcome at 2 years.
Kidokoro (316)	‘10	27 -32	12	aEEG to predict outcome	aEEG/EEG	Within 12 hrs	For 24 hours or more if abnormal	ND evaluation and Tumori Inage at 12 months	Absent cyclicity within 24h associated with poor outcome
Klebermass (341)	‘11	<30	143	aEEG to predict outcome	Single channel aEEG	First 2 weeks	Median duration of 250 minutes	3 year Bayley scales Assessment	aEEG has good predictive value for outcome
El-Dib (263)	‘11	<34	100 – 55 follow-up	aEEG to predict outcome	4-channel aEEG	Within 48 h after birth and at 1 week of life	Recorded for 12hours	4 months Bayley scales Assessment	Combination of aEEG and early CRUS at 1 week increased the sensitivity for detecting short-term adverse outcomes.
Wikstrom (315)	‘12	22-30	49	Early aEEG for prediction of long term outcome.	aEEG	During the first 72 hours of life.	Recorded for duration of 4 hours.	2 year Bayley scales Assessment	Outcome prediction accuracy of 75-80% at 24 hours & for the infants with no early indication of injury.
Vesoulis (314)	‘13	24 - 30	95	Evaluate association of seizures and neurodevelopmental outcome	aEEG	Once stable. Mean of 18.5 hours after birth	Recorded for an average duration of 66 hours.	2 year Bayley scales Assessment	High seizure burden associated with brain injury and mortality.

Reynolds (265)	'14	<30	136	Association between serial aEEG and outcome.	aEEG	Between the first 2 weeks of life and term	Recorded for duration of 4 hours.	2 year Bayley scales Assessment	Cyclicity and Burdjalov-scores in the first 6 weeks of life demonstrated associations with adverse outcome.
El-Dib (262)	'14	<34	84	Assess relationship between sleep and outcome	4-channel aEEG	At 34 weeks	12 hours	2 year Bayley scales Assessment	Association was evident between maturity of sleep (presence of cyclicity) and outcome at 9 months but not at 18 months.
Schwindt (479)	'15	<30 with small for GA	156 – 136 follow-up	Analyse the influence of being born small for gestational age on the aEEG	2 channel EEG	Within 2 weeks	10 minutes	2 year Bayley scales Assessment and a standardized neurological examination	Poorer aEEG and a poorer neurodevelopmental outcome at 24 months were evident in the infants that were small for GA compared to controls. Wikstrom et al aEEG scoring used.

Song (266)	‘15	<32	139	Could aEEG predict brain damage and long-term neurodevelopmental outcomes	aEEG	First 72 hours	4 – 24 hours	2 year Bayley scales Assessment	Severely abnormal aEEG (discontinuous activity and severely abnormal, without SWC, burst-suppression, flat trace, continuous low voltage electrographic seizures) has the potential to predict white matter damage.
Bruns (261)	‘17	<32	65	Compare the Hellström-Westas and Burdjalov aEEG classifications for outcome prediction	2 channel EEG	Within the first 72 h of life and	4 h within each day of life	2 year Bayley scales Assessment	Both classifications reported that sleep cycling was a valuable tool to assess survival.
Ralser (264)	‘17	<32	232	aEEG to predict outcome	2 channel EEG	No longer than 6 hours after birth	Duration of 72 hours	12 months Bayley scales Assessment	aEEG was predictive of outcome and the optimal period to record aEEG for the ability to predict outcome is within the first 2 days of life.

Weeke (328)	'17	<28	77	Relating EEG patterns to brain injury and outcome	2 channel EEG	Within the first 72 h of life	Unspecified	2 year Bayley scales Assessment	Rhythmic EEG patterns in extremely preterm infants may relate to head position/movement artefact therefore have a different significance. Periodic epileptiform discharges are common with an unclear significance, with no clear relation to brain injury or adverse cognitive outcome.
Middel (480)	'18	26 - 32	41 (first EEG and 43 (second)	aEEG to predict outcome	2 channel EEG	As soon as possible after birth and repeated at 1 week	Mean duration of 213 minutes	Neuropsychological tests assessing motor, cognitive and behavioural ability	The presence of aEEG cyclicity early after birth is a good sign of good cognitive outcome, however generally, aEEG is limited at predicting outcome

									in healthy preterm infants.
Hüning (481)	'18	<32	38	Study the use of aEEG and MRI combination for predicting outcome	2 channel EEG	Within the first 72 h of life	3 days. 4 hours of each day analysed.	2 year Bayley scales Assessment	Early postnatal aEEG, by using the Burdjalov scores, combined with cerebral MRI at term aids the prediction of neurodevelopmental outcome.
Burger (268)	'20	<32	306	Evaluate aEEG for predicting neurodevelopmental outcome	2 channel EEG	Within the first 72 h of life	Then at wk 1, wk 2, wk 3 & w4.	2 year Bayley scales Assessment	Burdjalov score showed differences in aEEG parametes, when regarding neurodevelopmental outcome. Especially psychomotor development.

APPENDIX F –

Publications that report incidence of seizures in preterm infants in the early postnatal period.

Author	*Year*	*Gestational Age*	*Study period*	*Number of infants*	*EEG / aEEG*	*Start of monitoring*	*Recording duration*	*Seizure incidence*
EEG								
Pisani (321)	'08	<36	Jan '99 – Dec '03	835 PT births in this period. (51 with seizures)	EEG	N/A	Monitoring of at least 60 minutes or until a complete cycle of states.	51 (6.1%) infants confirmed seizures.
Okumura (324)	'08	<37 Information provided for infants <32	'91 – '98	EEG obtained in 1045 of 1201 PT. (408 <32 weeks GA)	EEG	Mostly started within the first 72 hours of life	N/A	4 (0.9%) infants with confirmed seizures.
Glass (327)	'09	<34	Apr '98 – June '08	236 included in study	EEG	N/A	Minimum 2 hours or multiple recordings	3.8% infants with clinical seizures and 0.4% infants with confirmed.
Davis (326)	'10	<36 weeks	Jan '00 – Dec '05	6499 included in the study.	EEG	Discretion of clinician	N/A	414 (6.4%) infants had clinical seizures, of which 92 (22%) were confirmed by EEG

				414 had clinical seizures				
Pisani (325)	'12	24 - 32	Jan '00 – Dec '07	403 included in study	EEG	Discretion of clinician – infants that presented with risk factors or clinical signs suggestive of seizures	Recorded for at least an hour.	8.7% infants with confirmed seizures.
Le Bihannic (307)	'12	<30	Jan '01 – Aug '04	76 eligible – 61 included	EEG	In the first week of life, usually first 4 days Repeats at 31/32weeks and 36 weeks.	Mean duration 60 min (min 45 minutes)	2 (3%) infants with confirmed seizures.
aEEG								
Olischar (299)	'07	<30	Jan '00 – Dec '03	56 infants suffering from PIVH of any grade	aEEG	Within first 2 weeks of life (median day 6).	Weekly recordings of minimum of 4 hours	Electrographic seizures were evident in 50% of infants who had IVH grades I & II, and 75% of infants who had IVH grades III & IV.

Shah (318)	'10	<30	Apr '07 – June '08	51	aEEG	Monitoring within the first week of life; median age of 24 hours (range of 3 – 117 hours).	Median duration period of 74 hours (range of 12 – 140 hours)	11 (22%) infants with confirmed seizures.
West (305)	'11	<29	Mar '02 – Feb '04	76	2-channel EEG	EEG commenced within 48 hours of birth	Recorded for duration of 2 -12 hours. Analysis of 60 minutes.	5 (6.6%) infants with confirmed seizures.
Wikstrom (315)	'12	22 – 30	June '05 – May '07	49	aEEG	During the first 72 hours of life.	For 72 hours – 4 hour epochs analyised.	21 (43%) infants with confirmed seizures.
Vesoulis (314)	'13	24 - 30	'08 – '10	95	aEEG	EEG commenced as soon as the infant became stable, with an average age of 18.5 hours after birth	Recorded for an average duration of 66 hours.	46 (48%) infants with confirmed seizures